BMJ open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002280
Article Type:	Research
Date Submitted by the Author:	30-Oct-2012
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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Urology
Keywords:	NUTRITION & DIETETICS, Prostate disease < UROLOGY, PREVENTIVE MEDICINE

Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

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Text word count: 4413;
Abstract word count: 274;
Tables: 1; Figures: 6;
References: 60

Abstract

Background: ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer.

Objective: To conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Data Sources: MEDLINE and EMBASE were searched for relevant prospective and case-control studies.

Eligibility Criteria for Selecting Studies: We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates.

Design: Data were pooled using the generic inverse variance method with a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity was assessed by χ^2 and quantified by I^2.

Results: Data from 5 prospective and 7 case-control studies were pooled. The overall RR estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (1.08 [0.90 to 1.29], P=0.40, I^2=85%), but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies which became significant (0.91 [0.83 to 0.99], P=0.02) without evidence of heterogeneity (I^2=8%, P=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

Key Words: Alpha-linolenic acid, prostate cancer, omega-3 fatty acid, meta-analysis

Introduction

Prostate cancer is the second most common cancer in men worldwide [1]. Prostate cancer incidence rates vary widely among countries, populations, and races. Incidence rates vary by more than 25-fold worldwide, with the highest rates documented in the developed countries of North America, Europe, and Oceania, which may be due largely to the wide utilization of prostate- specific antigen (PSA) testing that detects clinically important tumors that might otherwise escape diagnosis [2]. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world [2], which is thought to reflect partly a difference in genetic susceptibility [3 4]. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [5-14]. In 1975, Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [12], and many studies have examined this connection [15-18], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [19-25]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs, since increased consumption of ω-3 fatty acids is advised for cardiovascular disease risk reduction [26-29] despite a possible association with prostate cancer [30].

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [31]. However, in the United States, the important sources of ALA are animal-based foods high in saturated fats, such as red meats, beef, pork, and lamb, rather than ALA-rich vegetable sources, such as walnuts. [25]. The largest proportion of ALA (53.8%) comes from red meat in Uruguay [32], but comes from margarine (25%) in the Netherlands [33]. Furthermore, foods such as bread, eggs, and margarine are now being enriched with ALA to increase their healthfulness. Therefore, it appears timely to determine whether there are associations between ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.

Methods

We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [34]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [35].

Study Selection

We conducted a search of MEDLINE (1948-April 17, 2009) and EMBASE (1974-April 17, 2009) using the following search terms and Boolean operators: *prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids)*. The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. This search strategy was last updated on August 28, 2012. We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. No lone abstracts or unpublished studies were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information about study design (prospective cohort, case-control, etc.), sample size and participant characteristics (nationality, race, named cohort, country of residence, gender, age, disease status, preexisting medical conditions), follow-up duration, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors, and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and where necessary by discussion with

another investigator (DJAJ). Authors were not contacted to request any additional information or translation.

Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. A pooled analysis of all reports was conducted using the Generic Inverse Variance method using random effects models [36] where the log RRs for cohort studies or log ORs for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer [30 37 38], ORs were considered as approximations of RRs. Inter-study heterogeneity was assessed by Cochrane's Q (Chi^2 $P<0.10$) and quantified by I^2 . An I^2 ≥50% indicated "substantial" heterogeneity and ≥75% indicated "considerable" heterogeneity. [39]. The influence of individual studies was investigated by systematically removing each study and recalculating the pooled effect. An *a priori* subgroup analysis by study design, (prospective versus case-control), was also undertaken to investigate heterogeneity. Meta-regressions were performed to assess the significance of study design on effect modification (STATA 11.2., College Station, USA). Publication bias was investigated by visual inspection of funnel plots, and formally tested using Begg's and Egger's tests.

Results

Search Results

Figure 1 shows the flow of the literature selection applying the systematic search and selection strategies to identify eligible reports. Two hundred and forty three reports were identified by the search and two reports were manually included after a database search. Of these, 233 were determined to be irrelevant on review of the titles and abstracts. Four additional reports were then manually included. The remaining 16 reports were retrieved and reviewed in full, of which 4 were excluded. Results for The Health Professionals' Follow-up Study were published in three separate publications at different times of follow-up [21 23 25]. Only the most

recent publication of the results, by Giovannucci et al. in 2007, was included in the analyses as representing the cumulative experience of the earlier assessments of this cohort [21]. A total of 12 reports, 5 prospective and 7 case-control studies, were included in the pooled analyses.

Study Characteristics

Table 1 shows the characteristics of the 12 included studies, which were composed of 7 case-control studies [32 40-45] and 5 prospective studies [19-22 24] that used 3 designs: cohort, nested case-cohort, and nested case-control. Five studies were conducted in North America, 1 in South America, and 6 in Europe. The 12 included studies contained a total of 14,795 cases of prostate cancer and 231,143 controls. All studies obtained dietary data using food frequency questionnaires (FFQ). Individual and average dietary ALA intake in these studies ranged from ≈0.05 to 4.16 g/d) and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

Primary Analysis

The overall analysis of the 12 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Seven studies reported a protective effect of ALA intake on prostate cancer, 2 of which were significant, and the remaining five studies reported a positive association, of which 3 were significant. Overall, although the relative risk was increased numerically by 8%, this increase in prostate cancer risk was not significant (RR: 1.08; 95%CI: 0.90, 1.29, P=0.40) (**Figure 2**). However, there was evidence of considerable inter-study heterogeneity (I^2=85%, P<0.00001). Systematic removal of each study during sensitivity analyses did not suggest any single study was an influential outlier.

Subgroup Analyses

In an *a priori* subgroup analysis, we found no evidence of effect measure modification according to study design (P for heterogeneity= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with substantial inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Removal of no single study during sensitivity analyses explained the heterogeneity. In prospective studies alone (n=5), no association between ALA intake and

prostate cancer risk was revealed (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 5**) but there existed considerable inter-study heterogeneity (I^2=69%, P=0.01) Sensitivity analyses showed that removal of the study by Giovannucci et al. [21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35 and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99, P=0.02) (**Figure 6**). Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al. [42] had an unusually large effect with a small standard error.

Discussion

Summary of Results

The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated heterogeneous effects of ALA on prostate cancer risk. Overall, there was no significant association between ALA intake and risk of prostate cancer. The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies by De Stefani et al. [32] and Ramon et al. [42], which reported large odds ratios greater than 3 but were still within 2 standard deviations of the mean effect, the association became weakly protective with decreased heterogeneity. When examining the prospective studies alone, the association between ALA intake and prostate cancer risk was weakly protective and after removal of the study by Giovannucci et al. [21] became significantly protective with no heterogeneity.

The results from the prospective studies are similar to those of previously published findings that examined only prospective studies [46]. Our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies and reached a similar conclusion although the case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain.

Variation in the Effect of ALA between Studies

In our study, different findings in the individual studies reviewed may be explained by a number of factors: variation in ALA consumption as a result of the population's dietary patterns,

differing sources of ALA, variation in ALA exposure levels, or use of different FFQs and food databases.

In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [33], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [25]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [20], but the most recent study from the United States reported a 25% increase in risk [21]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the "healthy" sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels.

In addition, in the case-control studies from Uruguay [32] and Spain [42] that showed the largest increases in prostate cancer risk demonstrated that meat, and not vegetable, was the major source of ALA. When these two studies were removed from the analysis of the case-control studies, the effect of ALA intake on prostate cancer changed from a weakly positive to a weakly protective effect. Compared with the other studies from Europe and the United States, there is a much higher consumption of meat in Spain [47] and Uruguay, with Uruguay having the highest meat consumption per capita in the world [48]. An earlier analysis of the Health Professionals Follow-up Study cohort [25] supports this positive association between red meat consumption and prostate cancer risk. Further, the two studies from Spanish-speaking countries also investigated the effect of animal fat on prostate cancer and both found significant positive associations. The Uruguayan study [32] observed an almost 3 times increased risk of prostate cancer at the highest level of ALA derived from animal sources and the Spanish study [42] revealed that the highest level of animal fat intake was associated with 2 times the risk of developing prostate cancer. These findings indicate that high meat intake rather than high ALA could explain ALA's apparent adverse effect on prostate cancer. A further explanation for the apparent association of prostate cancer incidence with vegetable sources of ALA may be that in addition those who follow healthy lifestyles with increased plant ALA sources may undergo more frequent prostate specific antigen (PSA) testing and therefore have early prostate cancer detection. In this respect

it has been found that higher whole grain intake was also associated with increased prostate cancer risk. However, when frequency of PSA screening was accounted for, the association of whole grains with prostate cancer incidence disappeared [49]. These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk.

Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and/or lower limits of ALA intake [21 32 43] and one study did not report numerical exposure levels [41]. Two studies, one from Spain [42] and one from the Netherlands [20], with the largest adequately defined upper and lower limits of ALA exposure ranges, paradoxically reported the second highest and the second lowest risk of developing prostate cancer, respectively. Since the studies with the greatest range of exposure do not necessarily show the greatest effects, dietary variation in the levels of exposure does not appear to explain differences among the studies, thereby making differences in dietary sources of ALA of more importance especially in relation to meat consumption in Western countries.

Lastly, in terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [50].

Overall Non-significant Effect of ALA

The overall effect of ALA on prostate cancer was found to be non-significant and may be attributed to a number of factors including ALA exposure levels that are within health guidelines, confounding from other polyunsaturated fatty acids, and the difference in effect of ALA on mortality versus incidence.

The mean dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [51], suggesting that ALA may not increase the risk of cancer more than any other nutrient which provides a stimulus to cell growth and since ALA

is a nutrient in which the Western diet is deficient [52], it may be that a deficiency prevents the growth of cancer rather than an excess causing prostate cancer growth.

Another issue to consider is confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) that might affect ALA metabolism [53] and consequently may introduce bias. The case-control study from the United States [45] demonstrated this as there was no significant association between ALA, omega-3, or omega-6 fatty acids and prostate cancer risk individually, but the highest dietary ratio of omega-6/omega-3 fatty acids was significantly associated with increased risk of high grade prostate cancer.

Finally, our analysis involved cancer incidence not mortality and ALA, and most other factors including energy intake, height, body mass index, calcium, and smoking are associated with cancer mortality [21]. The study by De Stefani et al. [32], which was the only study that defined cases solely as advanced prostate cancer, had the highest risk estimate of prostate cancer, indicating that ALA may be strongly associated with disease severity rather than incidence. In support of this point, the prospective study by Giovannucci et al. [21] found that higher ALA intake was more strongly associated with increased risk of fatal prostate cancer than with incident. However, three other prospective studies did not find any difference between the effects of ALA on incident or advanced prostate cancer cases [19 20 22]. From these mixed findings, it is unclear whether ALA is associated with severity of prostate cancer, but determining whether ALA impacts prostate cancer incidence or progression is an important distinction that should be investigated in the future. Furthermore, the picture of ALA's effect on prostate cancer is complicated by the positive association of incident prostate cancer with either serum or adipose tissue ALA levels [24 54-58] despite the in vitro evidence which suggests that ALA may suppress prostate cancer cell growth [59 60]. However, there appears to be some correlation between ALA intake and serum ALA levels. In terms of intake, Gann et al. [54] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to the prospective analysis from the Health Professionals Follow-Up Study [25], they found that red meat was positively associated with advanced prostate cancer, whereas diary foods were not. This corroboration not only suggests a correlation between ALA intake and serum ALA levels, but enforces the positive association between ALA from red meat and prostate cancer as seen in the studies from Uruguay [32] and Spain [42], rather than from plant foods.

Limitations and Possible Sources of Heterogeneity

In considering the limitations of the meta-analysis, it should be noted that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Interpretation of the analyses is complicated by the evidence of considerable heterogeneity among the studies, therefore a number of potential contributing factors should be considered. First, study design should be taken into account. The association between ALA intake and prostate cancer risk was stronger overall in the case-control studies than in the prospective. However, since case-control studies collect dietary intake information after disease development there is the possibility of recall bias, whereas prospective studies collect intake information before disease diagnosis. Secondly, follow-up time could also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from a non-significant to a significant positive association between ALA intake and prostate cancer. So, the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. After investigating this suggestion, the effect of follow-up duration on relative risk among the prospective studies was found to be positively, but not significantly correlated (r=0.47).

Conclusion

In conclusion, these findings provide no clear evidence of an association between dietary ALA intake and prostate cancer risk since studies that show an association between ALA intake and prostate cancer are observational and causation is difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional or inhibitory effect on prostate cancer risk and development. For the present, no significant association has been found and where any support of a positive effect was seen, red meat sources have been strongly implicated. The source of ALA appears to be of importance, particularly identifying whether it is from animal or vegetable sources, as ALA may be a marker for higher meat and fat intake in some countries both of which have been associated with increased prostate cancer risk. Attention should also be paid to the effect of ALA on

prostate cancer progression to address the issues of specific vulnerability identified in the studies of [21 32]. However, the relation of dietary intake of ALA to prostate cancer risk is likely to continue to be difficult to resolve through randomized controlled trials due to the significant public health implications of reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Of probably greater importance is determination of the sources of the fatty acid since ALA is associated in the North American diet with meat membranes and creamy salad dressings, which themselves may be markers of a suboptimal dietary pattern and lifestyle

Article Summary

Article Focus

- ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer
- A systematic review and meta-analysis of case-control and prospective studies was conducted to investigate the association between dietary ALA intake and prostate cancer risk

Key messages

- The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated overall no significant association between ALA intake and risk of prostate cancer
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies, which reported large odds ratios, the association became weakly protective with decreased heterogeneity
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity, which suggests an element of increased risk dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain

Strengths and Limitations:

- This meta-analysis includes both prospective and case control studies to determine the effect of ALA on prostate cancer

- Possible confounders and sources of heterogeneity were discussed and explored in relation to the results
- Interpretation of analyses was complicated by considerable heterogeneity among the studies, which may be due to lack of randomized controlled trials, study design, and follow-up duration

"What this Paper Adds"

ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. Although Carayol et al. conducted a meta-analysis on the effect of dietary ALA on prostate cancer in 2010, only prospective studies were analyzed and case-control studies were not included. Overall, we found no significant association between ALA intake and risk of prostate cancer. The results from the prospective studies were similar to those of previously published findings. However, the subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. The case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain. Additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional or inhibitory effect on prostate cancer risk and development.

Authorship

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of Contributors: AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS was involved in revising the article critically for important intellectual content. GE was involved

in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

Data Sharing

There is no additional data available.

Competing Interest Declaration

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) AJC, JLS, RS, GE, and DJAJ have not had financial support from any company for the submitted work; (2) AJC, JLS, RS, GE, and DJAJ have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) AJC, JLS, RS, GE, and DJAJ have no non-financial interests that may be relevant to the submitted work."

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [19]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.32	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.156†	0.82	0.41-1.65

* Prospective studies.

† Based on a 2000 kcal diet.

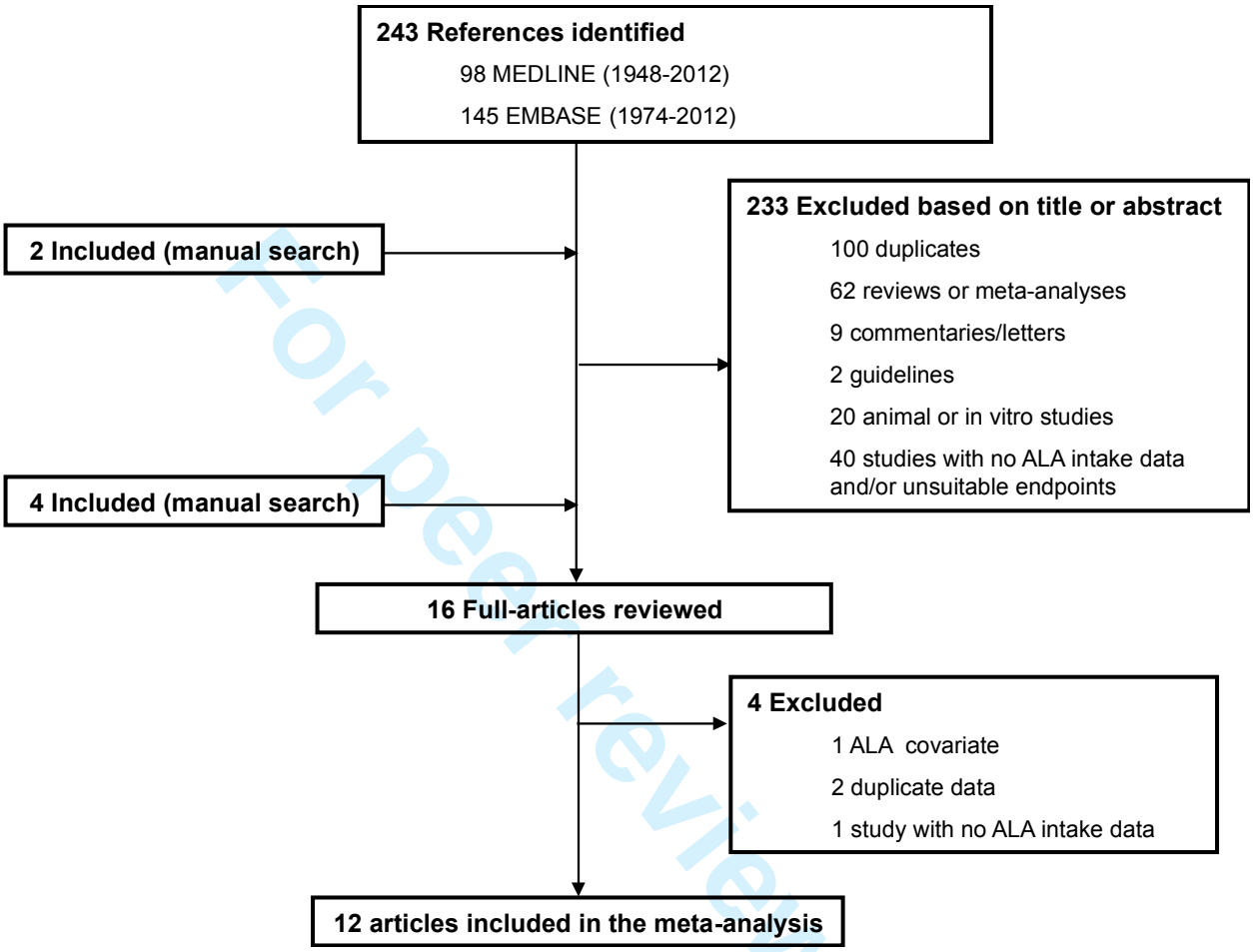

Figure 1 - Flow of the literature.

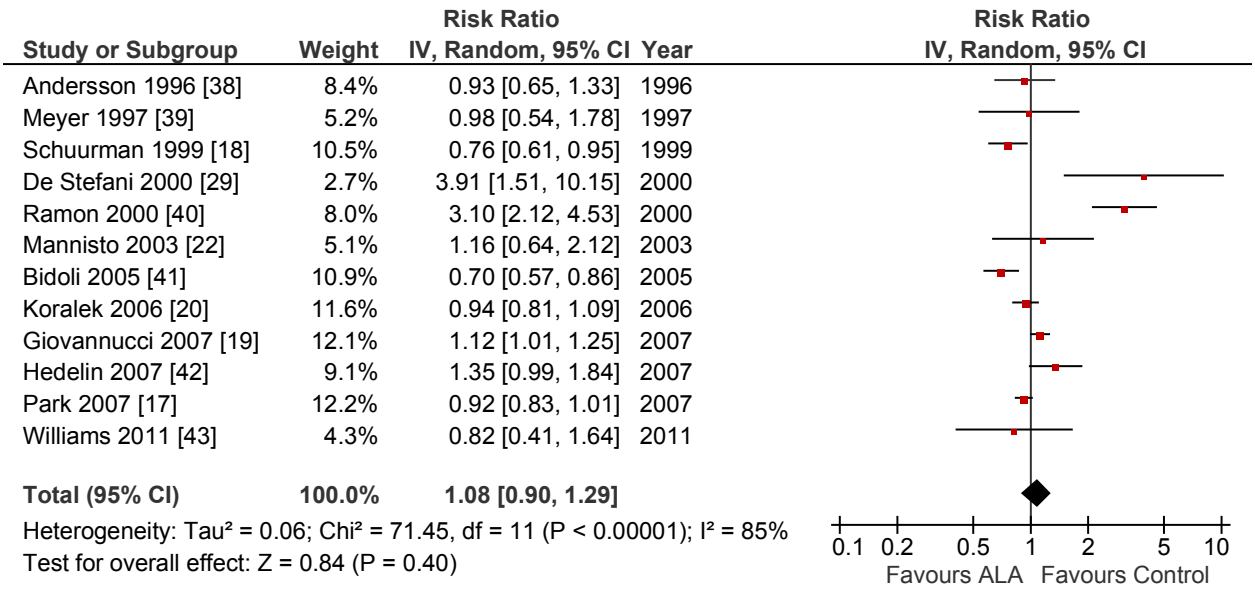

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].

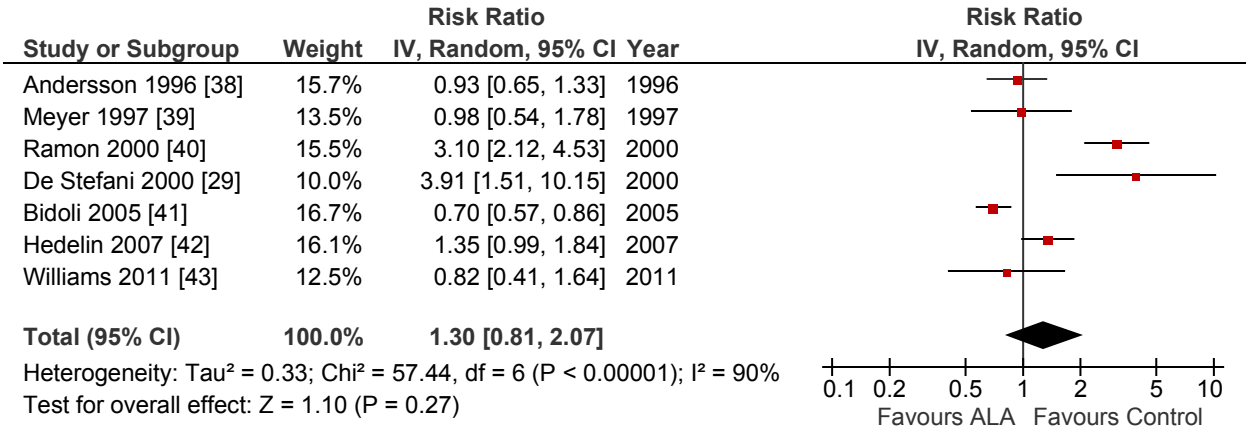

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated

using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].

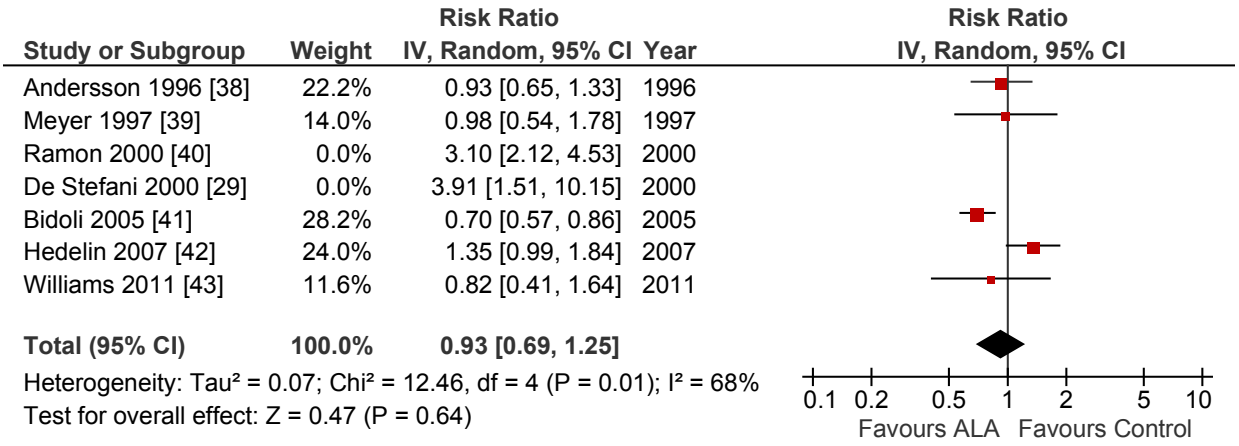

Figure 4 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by Ramon et al. [42] and De Stefani et al. [32] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].

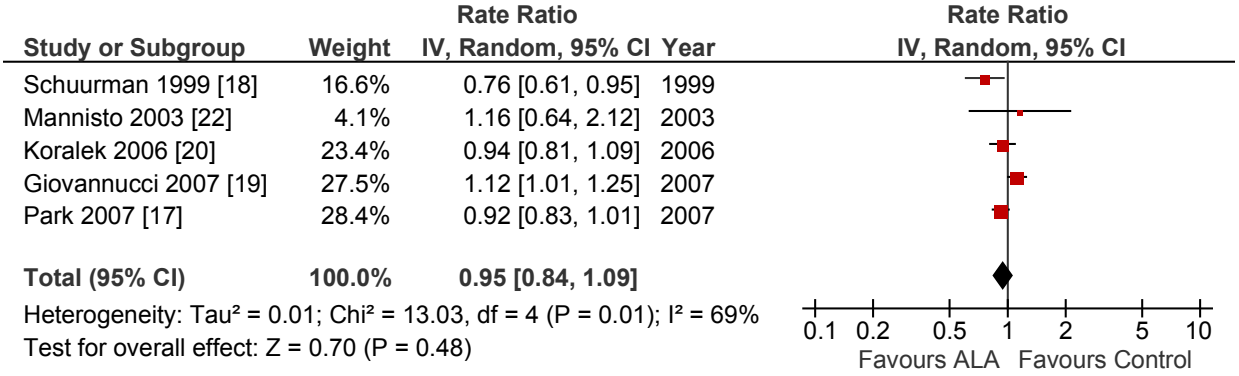

Figure 5 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane

Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].

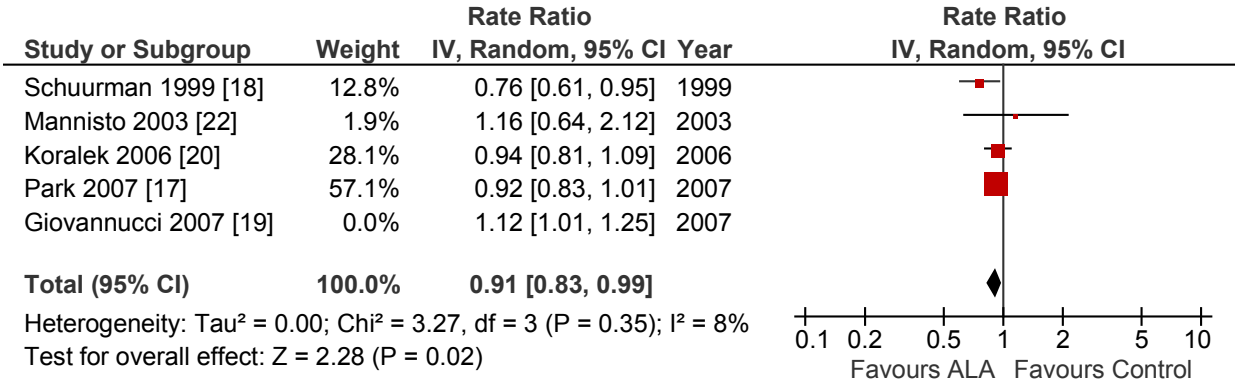

Figure 6 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [26]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.617 - 1.552	0.98	0.65-1.52
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/893 controls	≥65	-	-	-	0.98	0.64-1.76
Schuurman et al. 1999 [16]*	Netherlands	Nested case-cohort	58278 (1535 subcohort)	55-69	942	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-69	-	-	≥0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<80-80	-	-	0.72 - 2.1	3.1	2.3-4.7
Mannisto et al. 2005 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	248	5-8	1.0 - 2.5	1.16	0.84-2.15
Bidoli et al. 2006 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.8	0.7	0.6-0.8
Kolonel et al. 2006 [20]*	United States	Prospective cohort	29,092	65-74	1598	8.1	1.09 - 1.76	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.5	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [18]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.52	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.166†	0.82	0.41-1.65

* Prospective studies.

† Based on a 2000 kcal diet.

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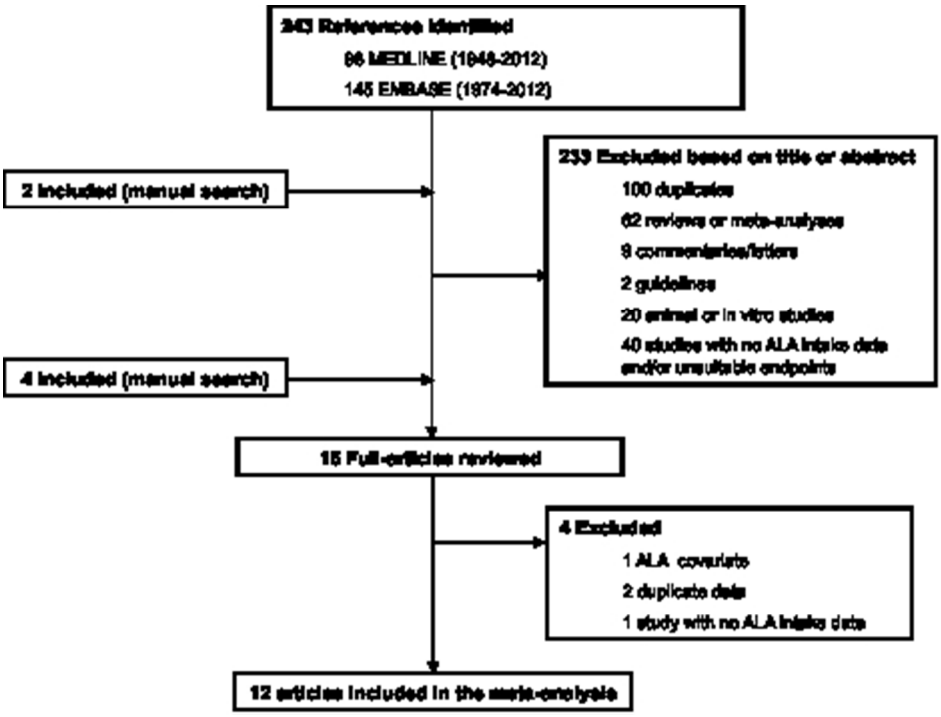

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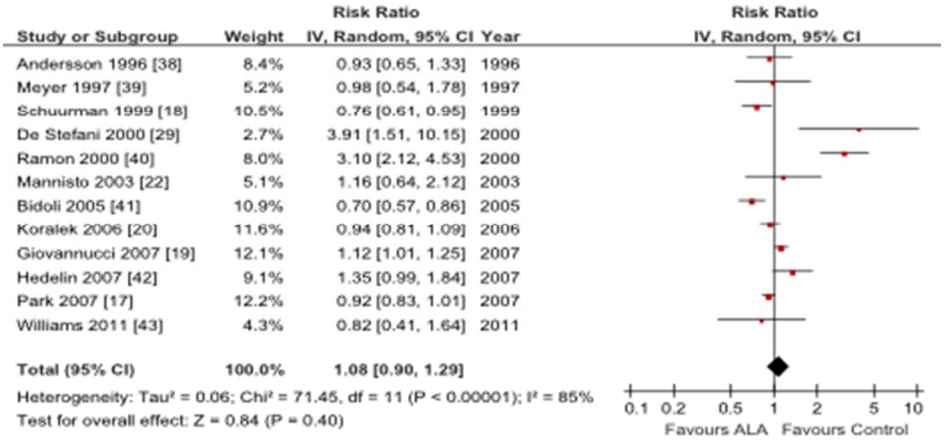

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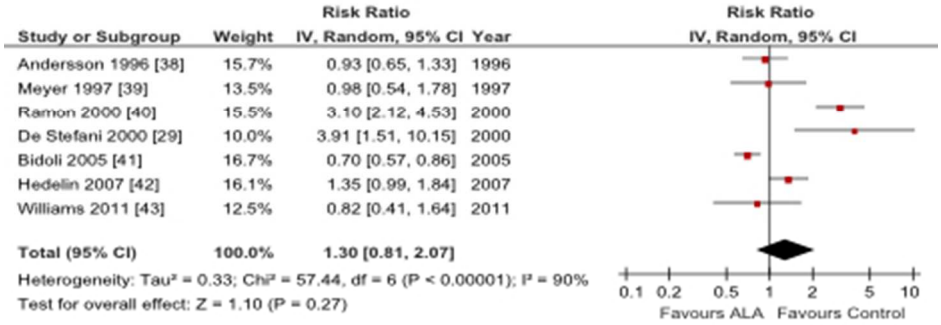

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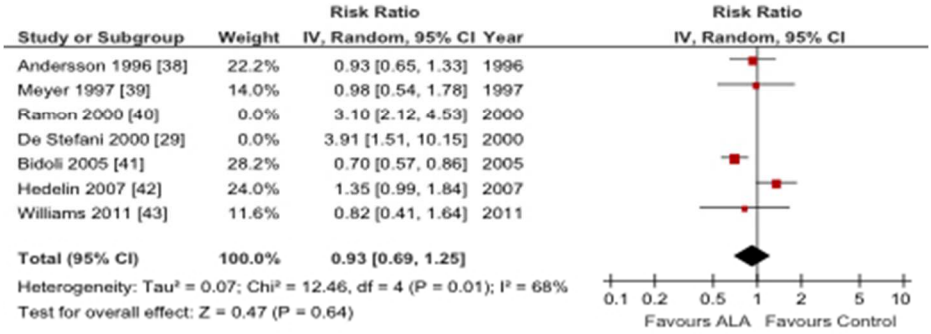

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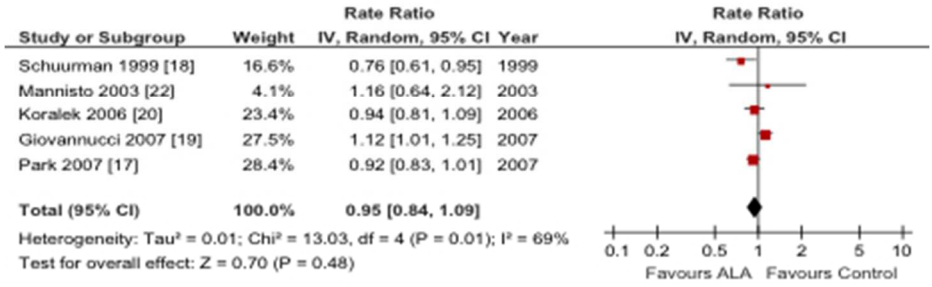

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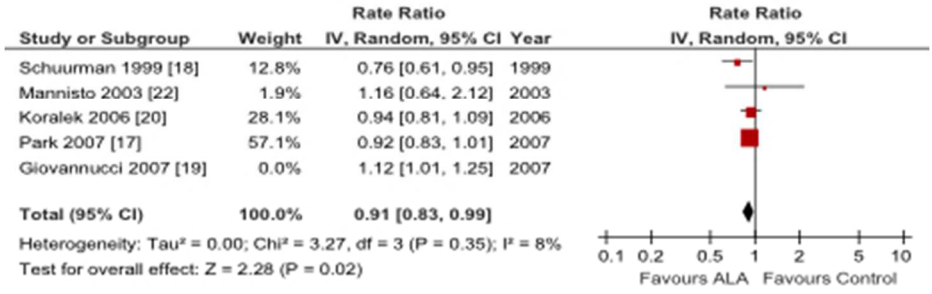

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BMJ open

Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002280.R1
Article Type:	Research
Date Submitted by the Author:	30-Jan-2013
Complete List of Authors:	Carleton, Amanda; University of Toronto, Faculty of Medicine; University of Toronto, Department of Nutritional Sciences Sievenpiper, John; University of Toronto, Department of Nutritional Sciences; St. Michael's Hospital, Risk Factor Modification Centre Jenkins, David; University of Toronto, Department of Nutritional Sciences; St. Michael's Hospital, Risk Factor Modification Centre de Souza, Russell; University of Toronto, Nutritional Sciences McKeown-Eyssen, Gail; University of Toronto, Department of Nutritional Sciences; University of Toronto, Dalla Lana School of Public Health
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Urology
Keywords:	NUTRITION & DIETETICS, Prostate disease < UROLOGY, PREVENTIVE MEDICINE

Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

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Text word count: 5184;
Abstract word count: 274;
Tables: 1; Figures: 7;
References: 74

ABSTRACT

Background: ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer.

Objective: To conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Data Sources: MEDLINE and EMBASE were searched for relevant prospective and case-control studies.

Eligibility Criteria for Selecting Studies: We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates.

Design: Data were pooled using the generic inverse variance method with a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity was assessed by χ^2 and quantified by I^2.

Results: Data from 5 prospective and 7 case-control studies were pooled. The overall RR estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (1.08 [0.90 to 1.29], P=0.40, I^2=85%), but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies became significant (0.91 [0.83 to 0.99], P=0.02) without evidence of heterogeneity (I^2=8%, P=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

Key Words: Alpha-linolenic acid, prostate cancer, omega-3 fatty acid, meta-analysis

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide [1]. Prostate cancer incidence rates vary widely among countries, populations, and races. Incidence rates vary by more than 25-fold worldwide, with the highest rates documented in the developed countries of North America, Europe, and Oceania, which may be due largely to the wide utilization of prostate- specific antigen (PSA) testing that detects clinically important tumors that might otherwise escape diagnosis [2]. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world [2], which is thought to reflect partly a difference in genetic susceptibility [3 4]. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [5-14]. In 1975, Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [12], and many studies have examined this connection [15-18], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [19-25]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs, since increased consumption of ω-3 fatty acids is advised for cardiovascular disease risk reduction [26-29] despite a possible association with prostate cancer [30].

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [31]. However, in the United States, the important sources of ALA are animal-based foods high in saturated fats, such as red meats, beef, pork, and lamb, rather than ALA-rich vegetable sources, such as walnuts. [25]. The largest proportion of ALA (53.8%) comes from red meat in Uruguay [32], but comes from margarine (25%) in the Netherlands [33]. Furthermore, foods such as bread, eggs, and margarine are now being enriched with ALA to increase their healthfulness.

There are currently divergent health views on ALA. Numerous epidemiological [34-39] and clinical studies [40-42] have shown that ALA is associated with a reduction in coronary heart disease (CHD) incidence and heart disease mortality. However, since ALA has also been associated with an increased risk of prostate cancer, [25 30 32 43-47] the seriousness of this potential

association requires that any favourable effects of ALA on CHD be weighed against its possible adverse effects on prostate cancer. Numerous prospective cohort [19-22 24] and case-control studies [32 45 48-52] have investigated the association between ALA and prostate cancer risk. While previous meta-analyses [30 53 54] have been conducted to determine whether a relationship exists, there has been no meta-analysis since 2010, examining the specific effect of dietary ALA on prostate cancer risk and none since 2009, that included in both prospective cohort and case-control studies. Therefore, it appears timely to determine whether there are associations between dietary ALA from ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.

METHODS

We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [55]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [56].

Study Selection

We conducted a search of MEDLINE (1948-April 17, 2009) and EMBASE (1974-April 17, 2009) using the following search terms and Boolean operators: *prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids)*. The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. This search strategy was last updated on August 28, 2012. We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. No lone abstracts or unpublished studies were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information about study design (prospective cohort, case-control, etc.), sample size and participant

characteristics (nationality, race, named cohort, country of residence, gender, age, disease status, preexisting medical conditions), follow-up duration, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors, and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and where necessary by discussion with another investigator (DJAJ). Authors were not contacted to request any additional information or translation.

Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. A pooled analysis of all reports was conducted using the Generic Inverse Variance method using random effects models [57] where the log RRs for cohort studies or log ORs for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer [30 54 58], ORs were considered as approximations of RRs. Since the initial risk of prostate cancer is low, it is unlikely that there will be a substantial discrepancy in approximating ORs to RRs. [59] Inter-study heterogeneity was assessed by Cochrane's Q (Chi^2 $P<0.10$) and quantified by I^2. An $I^2 \geq 50\%$ indicated "substantial" heterogeneity and $\geq 75\%$ indicated "considerable" heterogeneity. [60] Sources of heterogeneity were explored by sensitivity analyses whereby the influence of individual studies was investigated by systematic removal of each study followed by recalculation of the pooled effect estimate and heterogeneity, as well as removal of outlier studies with risk estimates larger than 2 standard deviations from the mean risk estimate and recalculation of the pooled effect estimate and heterogeneity. We also performed *a priori* subgroup analyses to assess effect modification by study design (prospective versus case-control). Post-hoc analyses included dichotomous subgroup analyses to assess effect

modification by study design (STATA 11.2., College Station, USA) and continuous analyses to assess the effect of the duration of follow-up on relative risk among prospective studies. Publication bias that was formally tested using Begg's and Egger's tests.

RESULTS

Search Results

Figure 1 shows the flow of the literature selection applying the systematic search and selection strategies to identify eligible reports. Two hundred and forty three reports were identified by the search and two reports were manually included after a database search. Of these, 233 were determined to be irrelevant on review of the titles and abstracts. Four additional reports were then manually included. The remaining 16 reports were retrieved and reviewed in full, of which 4 were excluded. Results for The Health Professionals' Follow-up Study were published in three separate publications at different times of follow-up [21 23 25]. Only the most recent publication of the results, by Giovannucci et al. in 2007, was included in the analyses as representing the cumulative experience of the earlier assessments of this cohort [21]. A total of 12 reports, 5 prospective and 7 case-control studies, were included in the pooled analyses.

Study Characteristics

Table 1 shows the characteristics of the 12 included studies, which were composed of 7 case-control studies [32 45 48-52] and 5 prospective studies [19-22 24] that used 3 designs: cohort, nested case-cohort, and nested case-control. Five studies were conducted in North America, 1 in South America, and 6 in Europe. The 12 included studies contained a total of 14,795 cases of prostate cancer and 231,143 controls. All studies obtained dietary data using food frequency questionnaires (FFQ). Individual and average dietary ALA intake in these studies ranged from ≈0.05 to 4.16 g/d) and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

Primary Analysis

The overall analysis of the 12 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Seven studies reported a protective effect of ALA intake on prostate cancer, one of which was significant, and the remaining five studies reported a

positive association, of which 3 were significant. Overall, although the relative risk was increased numerically by 8%, this increase in prostate cancer risk was not significant (RR: 1.08; 95%CI: 0.90, 1.29, P=0.40) (**Figure 2**). However, there was evidence of considerable inter-study heterogeneity (I^2=85%, P<0.00001). Systematic removal of each study during sensitivity analyses did not suggest any single study was an influential outlier.

Subgroup Analyses

Case-Control Studies

In an *a priori* meta-regression, we found no evidence of effect measure modification according to study design (P for heterogeneity= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with considerable inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Systematic removal of each individual study during sensitivity analyses did not explain the heterogeneity. Removal of the 2 case-control studies by Ramon et al.[45], De Stefani et al.[32] that reported risk estimates larger than 2 standard deviations from the pooled RR estimate reduced the inter-study heterogeneity (I^2=68%, P=0.01) but did not eliminate it (**Figure 4**). The overall association became weakly protective but was not significant (RR=0.93; 95%CI: 0.69,1.25, P=0.64) (**Figure 4**). Removal of the 3 case-control studies by Ramon et al.[45], De Stefani et al.[32], and Bidoli et al. [50] that had risk estimates outside the 95% CI of the pooled RR estimate, eliminated heterogeneity in the case-control studies (I^2=11%, P=0.34), but the overall non-significant association between ALA intake and prostate cancer risk remained (RR=1.08; 95%CI: 0.86,1.36, P=0.49) (**Figure 5**).

Prospective Studies

In prospective studies alone (n=5), no association between ALA intake and prostate cancer risk was revealed (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 6**) but there existed substantial inter-study heterogeneity (I^2=69%, P=0.01). Sensitivity analyses showed that removal of the study by Giovannucci et al. [21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35) and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99, P=0.02) (**Figure 7**). Duration of follow-up in prospective studies was found to be positively but not significantly associated with the magnitude of relative risk (r=0.47).

Publication Bias

Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al. [45] had an unusually large effect with a small standard error.

DISCUSSION

Summary of Results

The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated non-significant heterogeneous effects of ALA on prostate cancer risk. Overall, there was no significant association between ALA intake and risk of prostate cancer. The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies by De Stefani et al. [32] and Ramon et al. [45], which reported large odds ratios greater than 3 but were still within 2 standard deviations of the mean effect, the association became weakly protective with decreased heterogeneity. When examining the prospective studies alone, the association between ALA intake and prostate cancer risk was weakly protective and after removal of the study by Giovannucci et al. [21] became significantly protective with no heterogeneity.

The results from the prospective studies are similar to those of previously published findings that examined only prospective studies [53]. Our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies and reached a similar conclusion although the case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain.

Heterogeneity and the Effect of ALA between Studies

In our study, different findings reviewed and inter-study heterogeneity may be explained by a number of factors: variation in ALA consumption and sources of ALA as a result of the population's dietary patterns, variation in ALA exposure levels, use of different FFQs and food

databases, variation in adjustment factors, and difference in follow-up times among prospective studies.

Variation in ALA Consumption and Sources, and Population Dietary Patterns.

In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [33], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [25]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [20], but the most recent study from the United States reported a 25% increase in risk [21]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the "healthy" sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels [61 62].

In addition, in the case-control studies from Uruguay [32] and Spain [45] that showed the largest increases in prostate cancer risk demonstrated that meat, and not vegetable, was the major source of ALA. When these two studies were removed from the analysis of the case-control studies, the effect of ALA intake on prostate cancer changed from a weakly positive to a weakly protective effect. Compared with the other studies from Europe and the United States, there is a much higher consumption of meat in Spain [63] and Uruguay, with Uruguay having the highest meat consumption per capita in the world [64]. An earlier analysis of the Health Professionals Follow-up Study cohort [25] supports this positive association between red meat consumption and prostate cancer risk. Furthermore, the two studies from Spanish-speaking countries also investigated the effect of animal fat on prostate cancer and both found significant positive associations. The Uruguayan study [32] observed that at the highest level of ALA intake derived from animal sources resulted in almost 3 times the risk of developing prostate cancer and the Spanish study [45] revealed that the highest level of animal fat intake was associated with 2 times the risk. These findings indicate that high meat intake rather than high ALA may explain ALA's apparent adverse effect on prostate cancer. In further support of this idea, the study by Bidoli et al.[50] demonstrated a significant protective association between ALA and prostate cancer risk in

an Italian population where ALA is mainly derived from olive oil [65] and the diet is rich in raw vegetables [50] rather than meat, profiling an overall more "healthy" diet.

An explanation for the apparent association of prostate cancer incidence with vegetable sources of ALA may be that in addition those who follow healthy lifestyles with increased plant ALA sources may undergo more frequent prostate specific antigen (PSA) testing and therefore have early prostate cancer detection. In this respect it has been found that higher whole grain intake was also associated with increased prostate cancer risk. However, when frequency of PSA screening was accounted for, the association of whole grains with prostate cancer incidence disappeared [66]. These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk.

Variation in ALA Exposure Levels.

Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and/or lower limits of ALA intake [21 32 50] and one study did not report numerical exposure levels [49]. Two studies, one from Spain [45] and one from the Netherlands [20], with the largest adequately defined upper and lower limits of ALA exposure ranges, paradoxically reported the second highest and the second lowest risk of developing prostate cancer, respectively. Since the studies with the greatest range of exposure do not necessarily show the greatest effects, dietary variation in the levels of exposure does not appear to explain differences among the studies, thereby making differences in dietary sources of ALA of more importance especially in relation to meat consumption in Western countries.

Variation in FFQs and Food Databases.

In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [67].

Variation in Adjustment Factors.

Although all the studies reported adjusted RRs or ORs, the adjustment factors were not consistent among the studies. Some of the adjustment factors in these studies included age, smoking history, physical activity level, BMI, family history of prostate cancer, history of diabetes mellitus, race, education, socioeconomic status, area of residence and intakes of total calories, fat, processed meat, fish, lycopene, and vitamin E supplements. Currently, the most well-established risk factors for prostate cancer are age, family history of the disease, and race/ethnicity [68] and consequently are the most important adjustment factors. Only 4 [20-22 52] of the 12 included studies adjusted for all of these 3 factors. The studies conducted by Park et al. [19] and Mannisto et al. [24] did not adjust for age, which is by far the strongest predictor of prostate cancer incidence and death [68]. A family history of prostate cancer has been shown to increase the risk of diagnosis and death and this factor was not adjusted for in studies by Hedelin et al. [51], Andersson et al. [48], and Mannisto et al. [24] Race is a prostate cancer risk factor and prognostic factor, with African-American or Black men being at increased risk, and this was not adjusted for in the studies by Bidoli et al. [50], De Stefani et al. [32], Ramon et al. [45], and Meyer et al. [49] Differences in adjustment among the included studies, particularly with respect to the important factors of age, family history of prostate cancer, and race could result in differences in risk estimates, thereby contributing to inter-study heterogeneity.

Variation in Follow-up Duration.

Follow-up time may also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from a non-significant to a significant positive association between ALA intake and prostate cancer. So, the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. After investigating this suggestion, the effect of follow-up duration on relative risk among the prospective studies was found to be positively, but not significantly correlated (r=0.47).

Reasons for the Lack of Effect of ALA

The overall effect of ALA on prostate cancer was found to be non-significant but may result from a number of factors including ALA exposure levels that are within health guidelines, confounding from other polyunsaturated fatty acids, and the difference in effect of ALA on mortality versus incidence.

The mean dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [69], suggesting that ALA may not increase the risk of cancer more than any other nutrient promoting cell growth. Rather, since ALA is a nutrient deficient in the Western diet [70], it may be that a deficiency inhibits all cell growth, including tumour growth, instead of adequate or excess levels causing prostate cancer growth.

Another issue to consider is confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) that might affect ALA metabolism [71] and consequently may introduce bias. The case-control study from the United States [52] demonstrated this as there was no significant association between ALA, omega-3, or omega-6 fatty acids and prostate cancer risk individually, but the highest dietary ratio of omega-6/omega-3 fatty acids was significantly associated with increased risk of high grade prostate cancer.

Finally, our analysis involved cancer incidence rather than mortality and ALA, among other factors such as energy intake, height, body mass index, calcium, and smoking, are also associated with cancer mortality [21]. The study by De Stefani et al. [32], which was the only study that defined cases solely as advanced prostate cancer, had the highest risk estimate of prostate cancer, indicating that ALA may be strongly associated with disease severity rather than incidence. In support of this point, the prospective study by Giovannucci et al. [21] found that higher ALA intake was more strongly associated with increased risk of fatal prostate cancer than with incident. However, three other prospective studies did not find any difference between the effects of ALA on incident or advanced prostate cancer cases [19 20 22]. From these mixed findings, it is unclear whether ALA is associated with severity of prostate cancer, but determining whether ALA impacts prostate cancer incidence or progression is an important distinction that should be investigated in the future. Furthermore, the picture of ALA's effect on prostate cancer is complicated by the positive association of incident prostate cancer with either serum or adipose tissue ALA levels [24 43 44 46 47 72] despite the in vitro evidence which suggests that ALA may suppress prostate cancer cell growth [73 74]. However, there appears to be some correlation between

ALA intake and serum ALA levels. In terms of intake, Gann et al. [43] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to the prospective analysis from the Health Professionals Follow-Up Study [25], they found that red meat was positively associated with advanced prostate cancer, whereas diary foods were not. This corroboration not only suggests a correlation between ALA intake and serum ALA levels, but enforces the positive association between ALA from red meat and prostate cancer as seen in the studies from Uruguay [32] and Spain [45], rather than from plant foods.

Limitations

The first limitation of the meta-analysis is that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Second, interpretation of the analyses was complicated by the evidence of considerable heterogeneity among the studies, which as discussed above may have resulted from differences in ALA sources and population dietary patterns, ALA exposure levels, FFQs and food databases, adjustment factors, and duration of follow-up. There are also inherent limitations in the studies included based on study design. The association between ALA intake and prostate cancer risk was stronger overall in the case-control studies than in the prospective studies. However, there is the possibility of recall bias in case-control studies, as dietary intake information is collected after disease development.

CONCLUSION

In conclusion, these findings provide no clear evidence of an association between dietary ALA intake and prostate cancer risk. Further, since these observational studies can only show association between ALA intake and prostate cancer, possible causation would be difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, inhibitory, or no effect on prostate cancer risk and development. For the present, no significant association has been found and where any support of a positive effect was seen, red meat sources have been strongly implicated. The source of ALA appears to be of importance, particularly identifying whether it is from animal or vegetable sources, as ALA may be a marker for higher meat and fat intake in some countries both of which have been associated with increased prostate cancer risk. Attention should also be

paid to the effect of ALA on prostate cancer progression to address the issues of specific vulnerability identified in the studies of [21 32]. However, resolving the relation of dietary ALA to prostate cancer risk through randomized controlled trials will likely continue to be difficult due to the significant public health implications of reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Of probably greater importance is determination of the sources of the fatty acid since ALA is associated in the North American diet with meat membranes and creamy salad dressings, which themselves may be markers of a suboptimal dietary pattern and lifestyle

ARTICLE SUMMARY

Article Focus

- ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer
- A systematic review and meta-analysis of case-control and prospective studies was conducted to investigate the association between dietary ALA intake and prostate cancer risk

Key messages

- The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated overall no significant association between ALA intake and risk of prostate cancer
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies, which reported large odds ratios, the association became weakly protective but remained non-significant, with decreased heterogeneity
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity, which suggests an element of increased risk dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain

Strengths and Limitations:

- This meta-analysis includes both prospective and case control studies to determine the effect of ALA on prostate cancer

- Possible confounders and sources of heterogeneity were discussed and explored in relation to the results
- Interpretation of analyses was complicated by considerable heterogeneity among the studies, which may be due to lack of randomized controlled trials, variation in ALA sources and dietary patterns, variation in ALA exposure levels, differences in FFQs and food databases, variation in adjustment factors, follow-up duration, and study design

"What this Paper Adds"

ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. Although Carayol et al. conducted a meta-analysis on the effect of dietary ALA on prostate cancer in 2010, only prospective studies were analyzed and case-control studies were not included. Overall, we found no significant association between ALA intake and risk of prostate cancer. The results from the prospective studies were similar to those of previously published findings. However, the subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. The case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain. Additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, null, or inhibitory effect on prostate cancer risk and development.

AUTHORSHIP

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of Contributors: AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS

was involved in revising the article critically for important intellectual content. GE was involved in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

DATA SHARING

There is no additional data available.

COMPETING INTEREST DECLARATION

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) AJC, JLS, RS, and GE have not had financial support from any company for the submitted work; (2) AJC, JLS, RS, and GE have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) AJC, JLS, RS, and GE have no non-financial interests that may be relevant to the submitted work. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla,

Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg's, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. DJAJ's wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada.

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [19]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.32	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.156†	0.82	0.41-1.65

* Prospective studies.

† Based on a 2000 kcal diet.

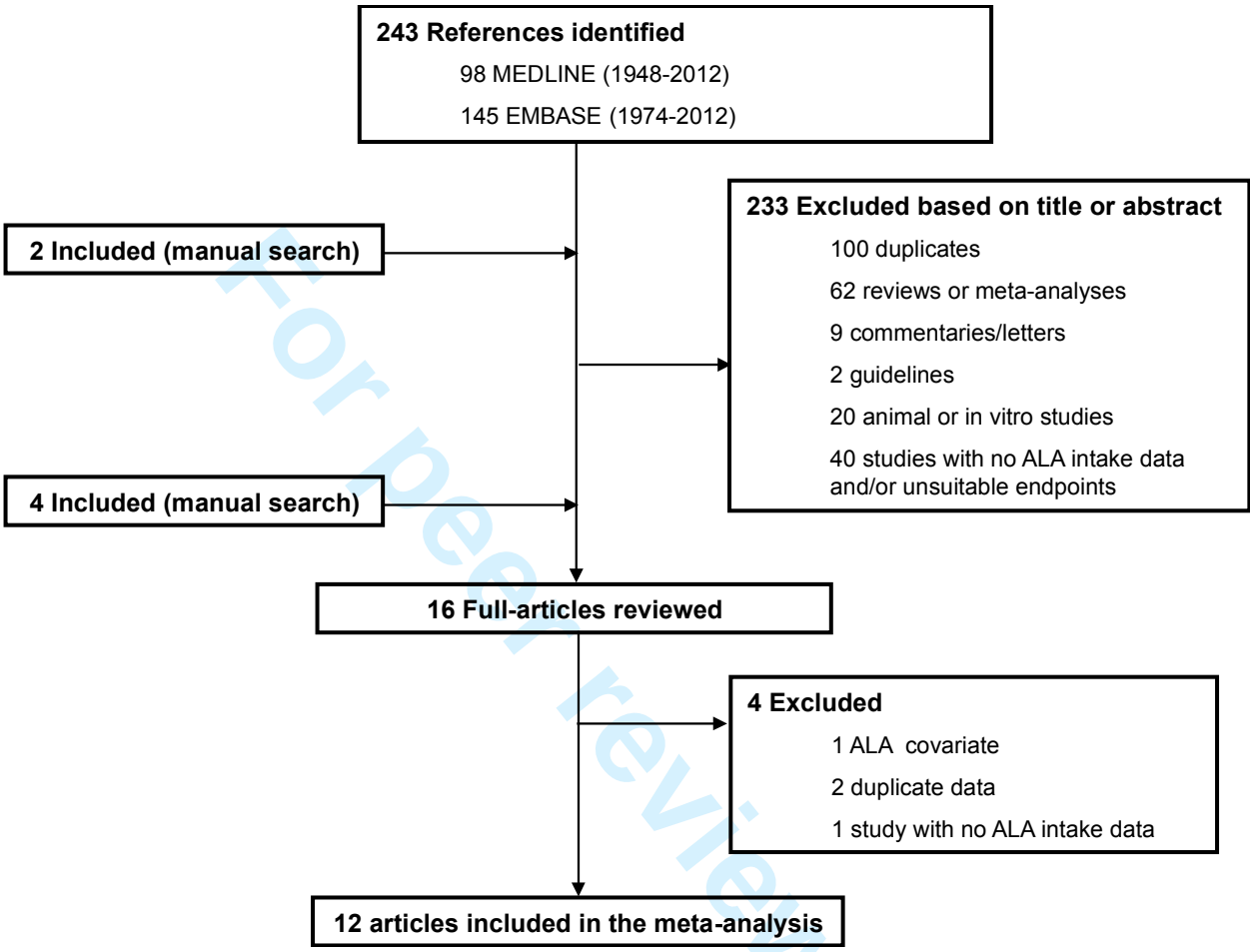

Figure 1 - Flow of the literature.

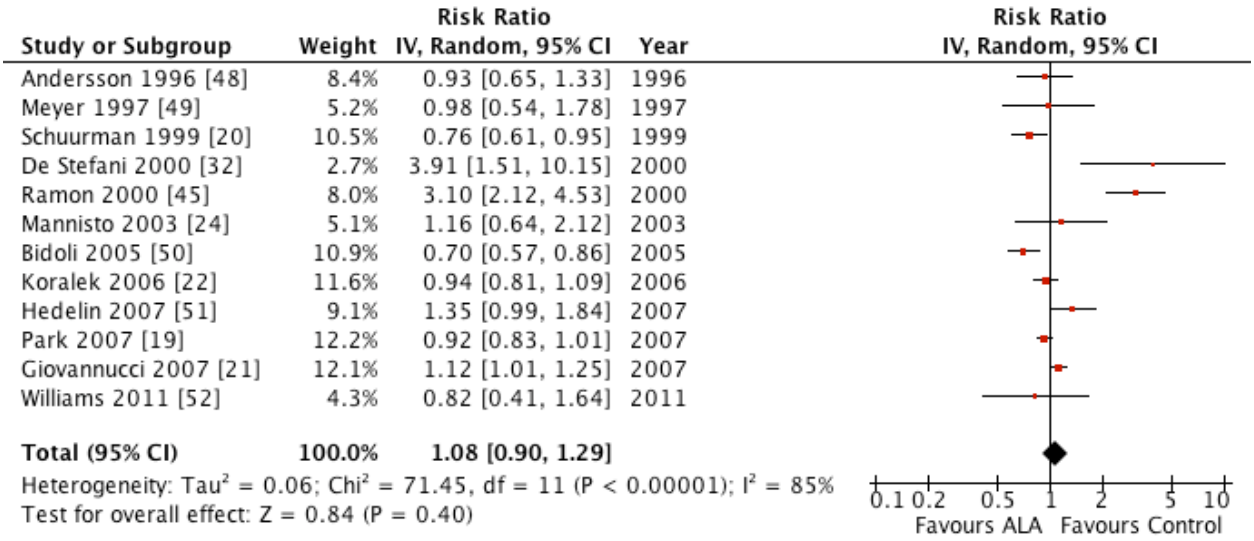

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

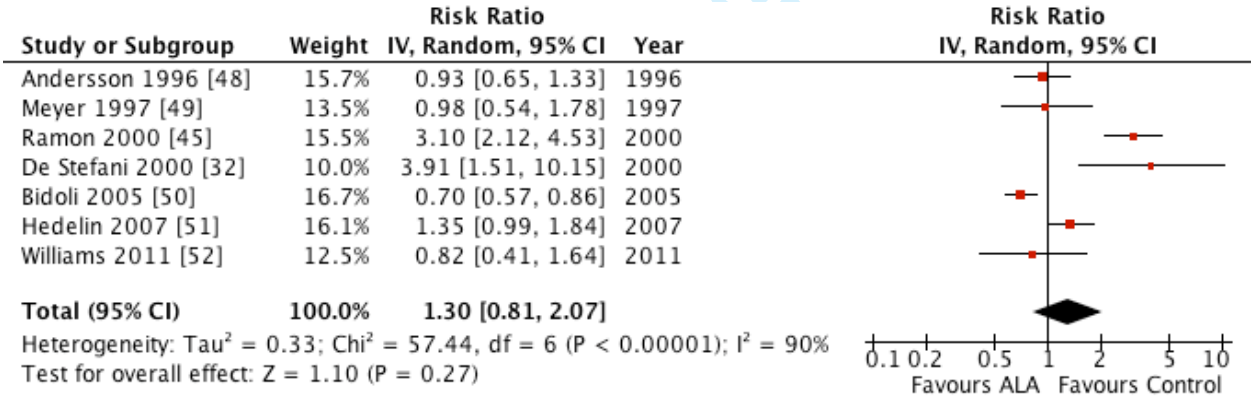

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a

significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

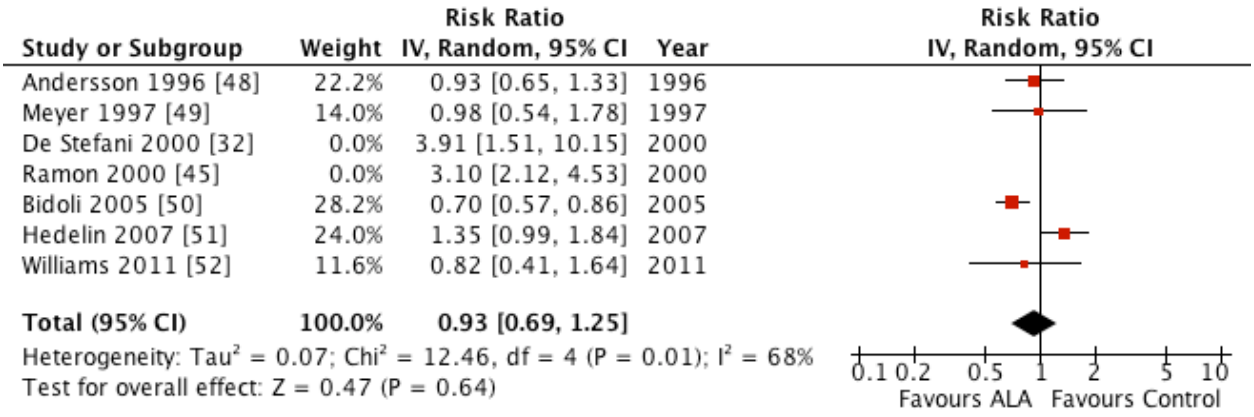

Figure 4 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by De Stefani et al. [32] and Ramon et al. [45] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

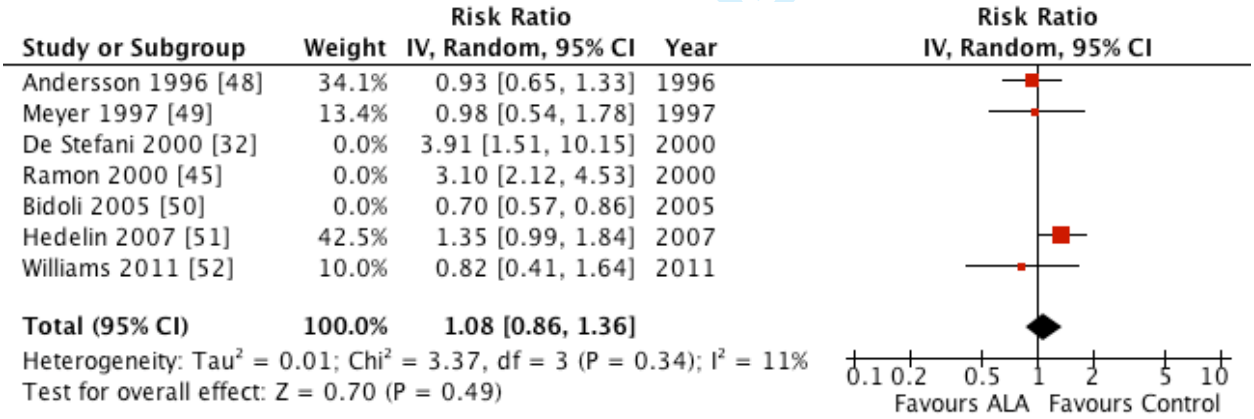

Figure 5 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by De Stefani et al. [32], Ramon et al. [45], and Bidoli et al. [50] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects

models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq$ 50 % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

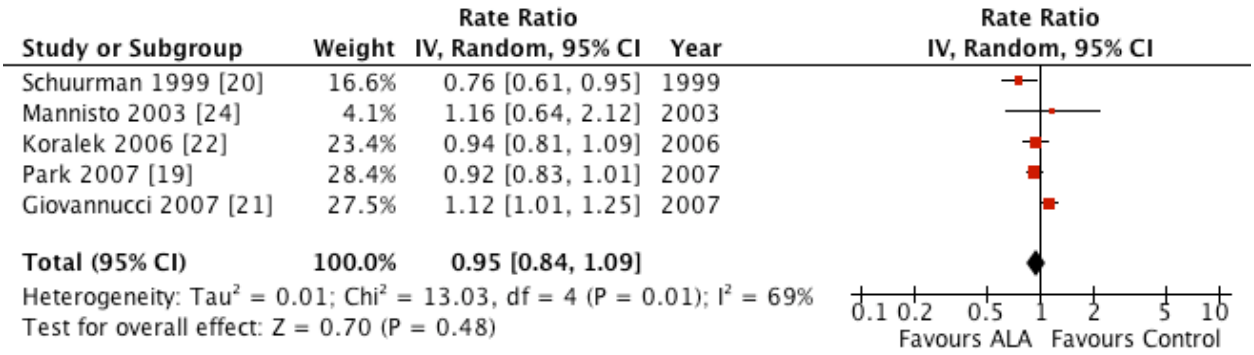

Figure 6 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq$ 50 % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

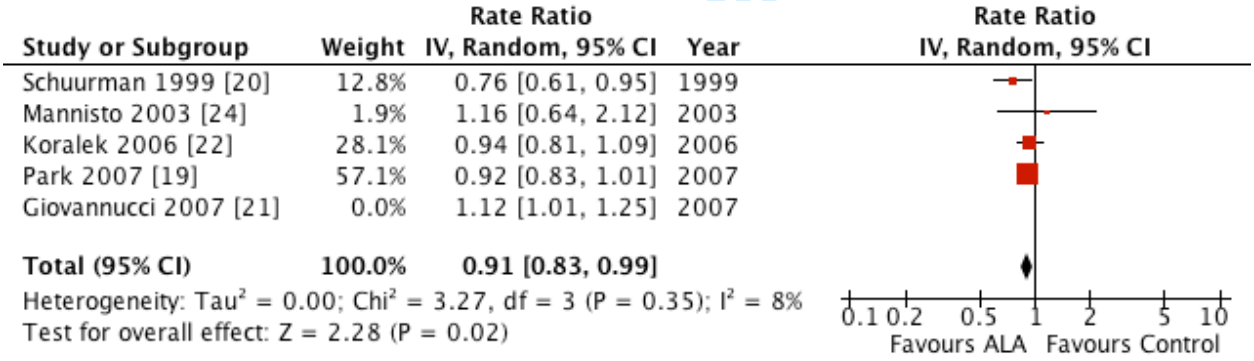

Figure 7 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a

significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

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Text word count: ~~4413~~5184;
Abstract word count: 274;
Tables: 1; Figures: ~~6;~~7;
References: ~~60~~74

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~~Abstract~~

ABSTRACT

Background: ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer.

Objective: To conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Data Sources: MEDLINE and EMBASE were searched for relevant prospective and case-control studies.

Eligibility Criteria for Selecting Studies: We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates.

Design: Data were pooled using the generic inverse variance method with a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity was assessed by χ^2 and quantified by I^2.

Results: Data from 5 prospective and 7 case-control studies were pooled. The overall RR estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (1.08 [0.90 to 1.29], P=0.40, I^2=85~~%);%),~~ but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies ~~which~~ became significant (0.91 [0.83 to 0.99], P=0.02) without evidence of heterogeneity (I^2=8%, P=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

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Key Words: Alpha-linolenic acid, prostate cancer, omega-3 fatty acid, meta-analysis

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~~Introduction~~INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide [1]. Prostate cancer incidence rates vary widely among countries, populations, and races. Incidence rates vary by more than 25-fold worldwide, with the highest rates documented in the developed countries of North America, Europe, and Oceania, which may be due largely to the wide utilization of prostate- specific antigen (PSA) testing that detects clinically important tumors that might otherwise escape diagnosis [2]. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world [2], which is thought to reflect partly a difference in genetic susceptibility [3 4]. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [5-14]. In 1975, Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [12], and many studies have examined this connection [15-18], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [19-25]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs, since increased consumption of ω-3 fatty acids is advised for cardiovascular disease risk reduction [26-29] despite a possible association with prostate cancer [30].

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [31]. However, in the United States, the important sources of ALA are animal-based foods high in saturated fats, such as red meats, beef, pork, and lamb, rather than ALA-rich vegetable sources, such as walnuts. [25]. The largest proportion of ALA (53.8%) comes from red meat in Uruguay [32], but comes from margarine (25%) in the Netherlands [33]. Furthermore, foods such as bread, eggs, and margarine are now being enriched with ALA to increase their healthfulness. ~~Therefore, it appears timely to determine whether there are associations between ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.~~

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~~Methods~~

~~We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [34]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [35].~~

There are currently divergent health views on ALA. Numerous epidemiological [34-39] and clinical studies [40-42] have shown that ALA is associated with a reduction in coronary heart disease (CHD) incidence and heart disease mortality. However, since ALA has also been associated with an increased risk of prostate cancer, [25 30 32 43-47] the seriousness of this potential association requires that any favourable effects of ALA on CHD be weighed against its possible adverse effects on prostate cancer. Numerous prospective cohort [19-22 24] and case-control studies [32 45 48-52] have investigated the association between ALA and prostate cancer risk. While previous meta-analyses [30 53 54] have been conducted to determine whether a relationship exists, there has been no meta-analysis since 2010, examining the specific effect of dietary ALA on prostate cancer risk and none since 2009, that included in both prospective cohort and case-control studies. Therefore, it appears timely to determine whether there are associations between dietary ALA from ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.

METHODS

We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [55]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [56].

Study Selection

We conducted a search of MEDLINE (1948-April 17, 2009) and EMBASE (1974-April 17, 2009) using the following search terms and Boolean operators: *prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids)*. The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. This search strategy was last updated on August 28, 2012. We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the

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incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. No lone abstracts or unpublished studies were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information about study design (prospective cohort, case-control, etc.), sample size and participant characteristics (nationality, race, named cohort, country of residence, gender, age, disease status, preexisting medical conditions), follow-up duration, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors, and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and where necessary by discussion with another investigator (DJAJ). Authors were not contacted to request any additional information or translation.

Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. A pooled analysis of all reports was conducted using the Generic Inverse Variance method using random effects models [~~36~~57] where the log RRs for cohort studies or log ORs for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer [30 ~~37~~54 ~~38~~58], ORs were considered as approximations of RRs. Since the initial risk of prostate cancer is low, it is unlikely that there will be a substantial discrepancy

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in approximating ORs to RRs. [59] Inter-study heterogeneity was assessed by Cochrane's Q (Chi^2 P<0.10) and quantified by I^2~~.~~. An I^2 ≥50% indicated "substantial" heterogeneity and ≥75% indicated "considerable" heterogeneity. [39]~~. The~~[60] Sources of heterogeneity were explored by sensitivity analyses whereby the influence of individual studies was investigated by ~~systematically removing~~systematic removal of each study ~~and recalculating~~followed by recalculation of the pooled effect~~. An *a*~~ estimate and heterogeneity, as well as removal of outlier studies with risk estimates larger than 2 standard deviations from the mean risk estimate and recalculation of the pooled effect estimate and heterogeneity. We also performed *a priori* subgroup ~~analysis~~ analyses to assess effect modification by study design~~,~~ (prospective versus case-control~~), was also undertaken to investigate heterogeneity. Meta-regressions were performed to assess the significance of~~). Post-hoc analyses included dichotomous subgroup analyses to assess effect modification by study design ~~on effect modification~~ (STATA 11.2., College Station, USA~~).~~) and continuous analyses to assess the effect of the duration of follow-up on relative risk among prospective studies. Publication bias ~~was investigated by visual inspection of funnel plots, and~~that was formally tested using Begg's and Egger's tests.

~~Results~~

RESULTS

Search Results

Figure 1 shows the flow of the literature selection applying the systematic search and selection strategies to identify eligible reports. Two hundred and forty three reports were identified by the search and two reports were manually included after a database search. Of these, 233 were determined to be irrelevant on review of the titles and abstracts. Four additional reports were then manually included. The remaining 16 reports were retrieved and reviewed in full, of which 4 were excluded. Results for The Health Professionals' Follow-up Study were published in three separate publications at different times of follow-up [21 23 25]. Only the most recent publication of the results, by Giovannucci et al. in 2007, was included in the analyses as representing the cumulative experience of the earlier assessments of this cohort [21]. A total of 12 reports, 5 prospective and 7 case-control studies, were included in the pooled analyses.

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Study Characteristics

Table 1 shows the characteristics of the 12 included studies, which were composed of 7 case-control studies [32 ~~40-45~~45 48-52] and 5 prospective studies [19-22 24] that used 3 designs: cohort, nested case-cohort, and nested case-control. Five studies were conducted in North America, 1 in South America, and 6 in Europe. The 12 included studies contained a total of 14,795 cases of prostate cancer and 231,143 controls. All studies obtained dietary data using food frequency questionnaires (FFQ). Individual and average dietary ALA intake in these studies ranged from ≈0.05 to 4.16 g/d) and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

Primary Analysis

The overall analysis of the 12 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Seven studies reported a protective effect of ALA intake on prostate cancer, ~~2~~one of which ~~were~~was significant, and the remaining five studies reported a positive association, of which 3 were significant. Overall, although the relative risk was increased numerically by 8%, this increase in prostate cancer risk was not significant (RR: 1.08; 95%CI: 0.90, 1.29, P=0.40) (**Figure 2**). However, there was evidence of considerable inter-study heterogeneity (I^2=85%, P<0.00001). Systematic removal of each study during sensitivity analyses did not suggest any single study was an influential outlier.

Subgroup Analyses

~~In an *a priori* subgroup analysis, we found no evidence of effect measure modification according to study design (P for heterogeneity= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with substantial inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Removal of no single study during sensitivity analyses explained the heterogeneity. In prospective studies alone (n=5), no association between ALA intake and prostate cancer risk was revealed (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 5**) but there existed considerable inter-study heterogeneity (I^2=69%, P=0.01). Sensitivity analyses showed that removal of the study by Giovannucci et al.[21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35 and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99,~~

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~~P=0.02) (**Figure 6**). Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al.[42] had an unusually large effect with a small standard error.~~

~~Discussion~~

Case-Control Studies

In an *a priori* meta-regression, we found no evidence of effect measure modification according to study design (P for heterogeneity= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with considerable inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Systematic removal of each individual study during sensitivity analyses did not explain the heterogeneity. Removal of the 2 case-control studies by Ramon et al.[45], De Stefani et al.[32] that reported risk estimates larger than 2 standard deviations from the pooled RR estimate reduced the inter-study heterogeneity (I^2=68%, P=0.01) but did not eliminate it (**Figure 4**). The overall association became weakly protective but was not significant (RR=0.93; 95%CI: 0.69,1.25, P=0.64) (**Figure 4**). Removal of the 3 case-control studies by Ramon et al.[45], De Stefani et al.[32], and Bidoli et al. [50] that had risk estimates outside the 95% CI of the pooled RR estimate, eliminated heterogeneity in the case-control studies (I^2=11%, P=0.34), but the overall non-significant association between ALA intake and prostate cancer risk remained (RR=1.08; 95%CI: 0.86,1.36, P=0.49) (**Figure 5**).

Prospective Studies

In prospective studies alone (n=5), no association between ALA intake and prostate cancer risk was revealed (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 6**) but there existed substantial inter-study heterogeneity (I^2=69%, P=0.01). Sensitivity analyses showed that removal of the study by Giovannucci et al. [21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35) and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99, P=0.02) (**Figure 7**). Duration of follow-up in prospective studies was found to be positively but not significantly associated with the magnitude of relative risk (r=0.47).

Publication Bias

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Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al. [45] had an unusually large effect with a small standard error.

DISCUSSION

Summary of Results

The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated non-significant heterogeneous effects of ALA on prostate cancer risk. Overall, there was no significant association between ALA intake and risk of prostate cancer. The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies by De Stefani et al. [32] and Ramon et al. [~~42~~45], which reported large odds ratios greater than 3 but were still within 2 standard deviations of the mean effect, the association became weakly protective with decreased heterogeneity. When examining the prospective studies alone, the association between ALA intake and prostate cancer risk was weakly protective and after removal of the study by Giovannucci et al. [21] became significantly protective with no heterogeneity.

The results from the prospective studies are similar to those of previously published findings that examined only prospective studies [~~46~~-53]. Our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies and reached a similar conclusion although the case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain.

~~*Variation in*~~ Heterogeneity and the Effect of ALA between Studies

In our study, different findings ~~in the individual studies~~ reviewed and inter-study heterogeneity may be explained by a number of factors: variation in ALA consumption and sources of ALA as a result of the population's dietary patterns, ~~differing sources of ALA,~~ variation in ALA exposure levels, ~~or~~ use of different FFQs and food databases, variation in adjustment factors, and difference in follow-up times among prospective studies.

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Variation in ALA Consumption and Sources, and Population Dietary Patterns.

In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [33], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [25]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [20], but the most recent study from the United States reported a 25% increase in risk [21]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the "healthy" sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels~~.~~ [61 62].

In addition, in the case-control studies from Uruguay [32] and Spain [42~~45~~] that showed the largest increases in prostate cancer risk demonstrated that meat, and not vegetable, was the major source of ALA. When these two studies were removed from the analysis of the case-control studies, the effect of ALA intake on prostate cancer changed from a weakly positive to a weakly protective effect. Compared with the other studies from Europe and the United States, there is a much higher consumption of meat in Spain [47~~63~~] and Uruguay, with Uruguay having the highest meat consumption per capita in the world [48~~.~~ 64]. An earlier analysis of the Health Professionals Follow-up Study cohort [25] supports this positive association between red meat consumption and prostate cancer risk. ~~Further~~Furthermore, the two studies from Spanish-speaking countries also investigated the effect of animal fat on prostate cancer and both found significant positive associations. The Uruguayan study [32] observed ~~an almost 3 times increased risk of prostate cancer at the highest level of ALA derived from animal sources and the Spanish study [42] revealed that the highest level of animal fat intake was associated with 2 times the risk of developing prostate cancer. These findings indicate that high meat intake rather than high ALA could explain ALA's apparent adverse effect on prostate cancer. A further~~that at the highest level of ALA intake derived from animal sources resulted in almost 3 times the risk of developing prostate cancer and the Spanish study [45] revealed that the highest level of animal fat intake was associated with 2 times the risk. These findings indicate that high meat intake rather than high ALA may explain ALA's apparent adverse effect on prostate cancer. In further support of this

idea, the study by Bidoli et al.[50] demonstrated a significant protective association between ALA and prostate cancer risk in an Italian population where ALA is mainly derived from olive oil [65] and the diet is rich in raw vegetables [50] rather than meat, profiling an overall more "healthy" diet.

An explanation for the apparent association of prostate cancer incidence with vegetable sources of ALA may be that in addition those who follow healthy lifestyles with increased plant ALA sources may undergo more frequent prostate specific antigen (PSA) testing and therefore have early prostate cancer detection. In this respect it has been found that higher whole grain intake was also associated with increased prostate cancer risk. However, when frequency of PSA screening was accounted for, the association of whole grains with prostate cancer incidence disappeared [49] ~~.~~ [66] . These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk.

Variation in ALA Exposure Levels.

Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and/or lower limits of ALA intake [21 32 ~~43~~50 ~~and one study did not report numerical exposure levels [41] .~~ ~~Two studies, one from Spain [42]~~ and one study did not report numerical exposure levels [49]. Two studies, one from Spain [45] and one from the Netherlands [20], with the largest adequately defined upper and lower limits of ALA exposure ranges, paradoxically reported the second highest and the second lowest risk of developing prostate cancer, respectively. Since the studies with the greatest range of exposure do not necessarily show the greatest effects, dietary variation in the levels of exposure does not appear to explain differences among the studies, thereby making differences in dietary sources of ALA of more importance especially in relation to meat consumption in Western countries.

~~Lastly, in~~

Variation in FFQs and Food Databases.

In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food

databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [~~5067~~].

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Variation in Adjustment Factors.

Although all the studies reported adjusted RRs or ORs, the adjustment factors were not consistent among the studies. Some of the adjustment factors in these studies included age, smoking history, physical activity level, BMI, family history of prostate cancer, history of diabetes mellitus, race, education, socioeconomic status, area of residence and intakes of total calories, fat, processed meat, fish, lycopene, and vitamin E supplements. Currently, the most well-established risk factors for prostate cancer are age, family history of the disease, and race/ethnicity [68] and consequently are the most important adjustment factors. Only 4 [20-22 52] of the 12 included studies adjusted for all of these 3 factors. The studies conducted by Park et al. [19] and Mannisto et al. [24] did not adjust for age, which is by far the strongest predictor of prostate cancer incidence and death [68]. A family history of prostate cancer has been shown to increase the risk of diagnosis and death and this factor was not adjusted for in studies by Hedelin et al. [51], Andersson et al. [48], and Mannisto et al. [24] Race is a prostate cancer risk factor and prognostic factor, with African-American or Black men being at increased risk, and this was not adjusted for in the studies by Bidoli et al. [50], De Stefani et al. [32], Ramon et al. [45], and Meyer et al. [49] Differences in adjustment among the included studies, particularly with respect to the important factors of age, family history of prostate cancer, and race could result in differences in risk estimates, thereby contributing to inter-study heterogeneity.

Variation in Follow-up Duration.

Follow-up time may also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from a non-significant to a significant positive association between ALA intake and prostate cancer. So, the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. After investigating this suggestion, the effect of follow-up duration on relative risk among the prospective studies was found to be positively, but not significantly correlated (r=0.47).

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~~***Overall Non-significant***~~

Reasons for the Lack of Effect of ALA

The overall effect of ALA on prostate cancer was found to be non-significant ~~and~~but may ~~be attributed to~~result from a number of factors including ALA exposure levels that are within health guidelines, confounding from other polyunsaturated fatty acids, and the difference in effect of ALA on mortality versus incidence.

The mean dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [~~51~~69], suggesting that ALA may not increase the risk of cancer more than any other nutrient ~~which provides a stimulus to~~promoting cell growth ~~and~~. Rather, since ALA is a nutrient deficient in ~~which~~ the Western diet ~~is deficient~~ [~~52~~70], it may be that a deficiency ~~prevents the~~inhibits all cell growth, including tumour growth, instead of ~~cancer rather than an~~adequate or excess levels causing prostate cancer growth.

Another issue to consider is confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) that might affect ALA metabolism [~~53~~71] and consequently may introduce bias. The case-control study from the United States [~~45~~52] demonstrated this as there was no significant association between ALA, omega-3, or omega-6 fatty acids and prostate cancer risk individually, but the highest dietary ratio of omega-6/omega-3 fatty acids was significantly associated with increased risk of high grade prostate cancer.

Finally, our analysis involved cancer incidence ~~not~~rather than mortality and ALA, ~~and most~~among other factors ~~including~~such as energy intake, height, body mass index, calcium, and smoking, are also associated with cancer mortality [21]. The study by De Stefani et al. [32], which was the only study that defined cases solely as advanced prostate cancer, had the highest risk estimate of prostate cancer, indicating that ALA may be strongly associated with disease severity rather than incidence. In support of this point, the prospective study by Giovannucci et al. [21] found that higher ALA intake was more strongly associated with increased risk of fatal prostate cancer than with incident. However, three other prospective studies did not find any difference between the effects of ALA on incident or advanced prostate cancer cases [19 20 22]. From these mixed findings, it is unclear whether ALA is associated with severity of prostate cancer, but determining whether ALA impacts prostate cancer incidence or progression is an important

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distinction that should be investigated in the future. Furthermore, the picture of ALA's effect on prostate cancer is complicated by the positive association of incident prostate cancer with either serum or adipose tissue ALA levels [24 ~~54 58~~43 44 46 47 72] despite the in vitro evidence which suggests that ALA may suppress prostate cancer cell growth [~~59 60~~73 74]. However, there appears to be some correlation between ALA intake and serum ALA levels. In terms of intake, Gann et al. [~~54~~43] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to the prospective analysis from the Health Professionals Follow-Up Study [25], they found that red meat was positively associated with advanced prostate cancer, whereas diary foods were not. This corroboration not only suggests a correlation between ALA intake and serum ALA levels, but enforces the positive association between ALA from red meat and prostate cancer as seen in the studies from Uruguay [32] and Spain [~~42~~45], rather than from plant foods.

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Limitations ~~***and Possible Sources of Heterogeneity***~~

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~~In considering the limitations~~The first limitation of the meta-analysis~~, it should be noted~~ is that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. ~~Interpretation~~Second, interpretation of the analyses ~~is~~was complicated by the evidence of considerable heterogeneity among the studies, ~~therefore a number of potential contributing~~which as discussed above may have resulted from differences in ALA sources and population dietary patterns, ALA exposure levels, FFQs and food databases, adjustment factors ~~should be considered. First~~, and duration of follow-up. There are also inherent limitations in the studies included based on study design ~~should be taken into account.~~. The association between ALA intake and prostate cancer risk was stronger overall in the case-control studies than in the prospective~~. However, since case-control studies collect dietary intake information after disease development there is the possibility of recall bias, whereas prospective studies collect intake information before disease diagnosis. Secondly, follow-up time could~~ studies. However, there is the possibility of recall bias in case-control studies, as dietary intake information is collected after disease development~~ also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from

~~a non-significant to a significant positive association between ALA intake and prostate cancer. So, the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. After investigating this suggestion, the effect of follow-up duration on relative risk among the prospective studies was found to be positively, but not significantly correlated (r=0.47).~~

~~Conclusion~~

CONCLUSION

In conclusion, these findings provide no clear evidence of an association between dietary ALA intake and prostate cancer risk. Further, since these observational studies ~~that~~can only show ~~an~~ association between ALA intake and prostate cancer ~~are observational and~~, possible causation ~~is~~would be difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional ~~or~~ , inhibitory, or no effect on prostate cancer risk and development. For the present, no significant association has been found and where any support of a positive effect was seen, red meat sources have been strongly implicated. The source of ALA appears to be of importance, particularly identifying whether it is from animal or vegetable sources, as ALA may be a marker for higher meat and fat intake in some countries both of which have been associated with increased prostate cancer risk. Attention should also be paid to the effect of ALA on prostate cancer progression to address the issues of specific vulnerability identified in the studies of [21 32]. However, resolving the relation of dietary ~~intake of~~ ALA to prostate cancer risk ~~is likely to continue to be difficult to resolve~~ through randomized controlled trials will likely continue to be difficult due to the significant public health implications of reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Of probably greater importance is determination of the sources of the fatty acid since ALA is associated in the North American diet with meat membranes and creamy salad dressings, which themselves may be markers of a suboptimal dietary pattern and lifestyle.

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~~Article Summary~~

ARTICLE SUMMARY

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Article Focus

- ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer
- A systematic review and meta-analysis of case-control and prospective studies was conducted to investigate the association between dietary ALA intake and prostate cancer risk

Key messages

- The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated overall no significant association between ALA intake and risk of prostate cancer
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies, which reported large odds ratios, the association became weakly protective but remained non-significant, with decreased heterogeneity
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity, which suggests an element of increased risk dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain

Strengths and Limitations:

- This meta-analysis includes both prospective and case control studies to determine the effect of ALA on prostate cancer
- Possible confounders and sources of heterogeneity were discussed and explored in relation to the results
- Interpretation of analyses was complicated by considerable heterogeneity among the studies, which may be due to lack of randomized controlled trials, ~~study design, and follow-up duration~~variation in ALA sources and dietary patterns, variation in ALA exposure levels, differences in FFQs and food databases, variation in adjustment factors, follow-up duration, and study design

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"What this Paper Adds"

ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. Although Carayol et al. conducted a meta-analysis on the effect of dietary ALA on prostate cancer in 2010, only prospective studies were analyzed and case-control studies were not included. Overall, we found no significant association between ALA intake and risk of prostate cancer. The results from the prospective studies were similar to those of previously published findings. However, the subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. The case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain. Additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, null, or inhibitory effect on prostate cancer risk and development.

~~Authorship~~

AUTHORSHIP

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of Contributors: AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS was involved in revising the article critically for important intellectual content. GE was involved in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

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~~Data Sharing~~

DATA SHARING

There is no additional data available.

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~~Competing Interest Declaration~~

COMPETING INTEREST DECLARATION

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) AJC, JLS, RS, ~~GE,~~ and ~~DJAJ~~GE have not had financial support from any company for the submitted work; (2) AJC, JLS, RS, ~~GE,~~ and ~~DJAJ~~GE have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) AJC, JLS, RS, ~~GE, and DJAJ have no non-financial interests that may be relevant to the submitted work."~~and GE have no non-financial interests that may be relevant to the submitted work. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla,

Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg's, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. DJAJ's wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada.

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95%CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [19]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.32	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.156†	0.82	0.41-1.65

* Prospective studies.

† Based on a 2000 kcal diet.

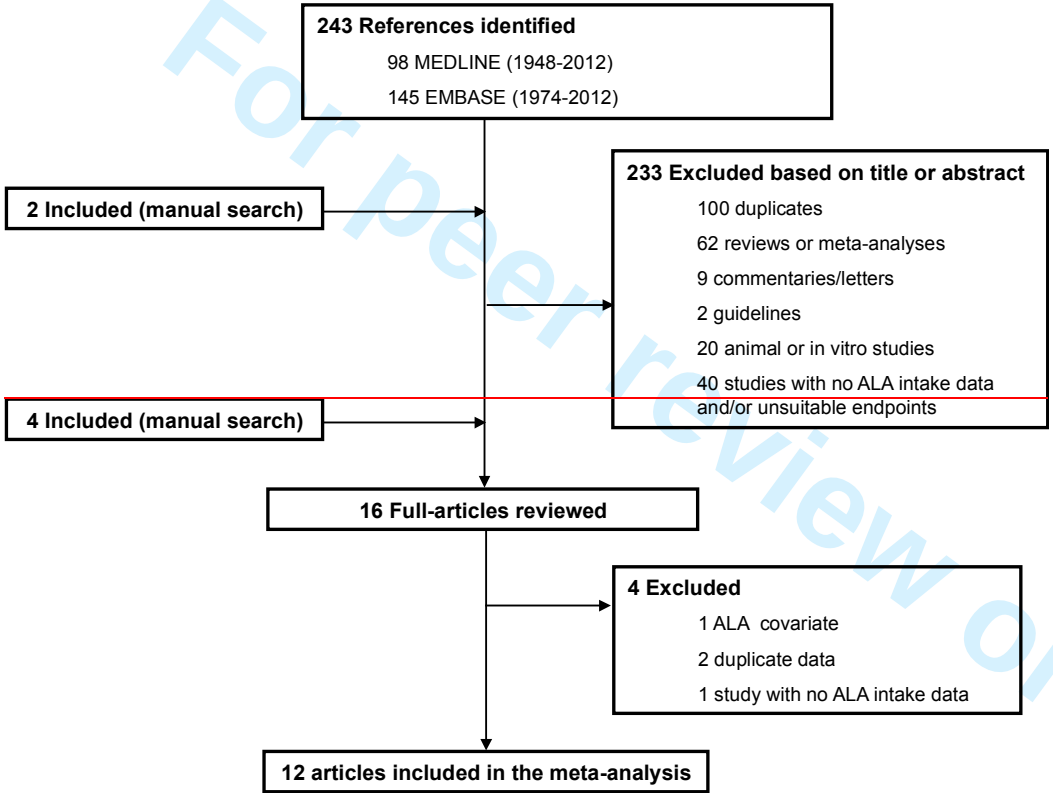

Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95%CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
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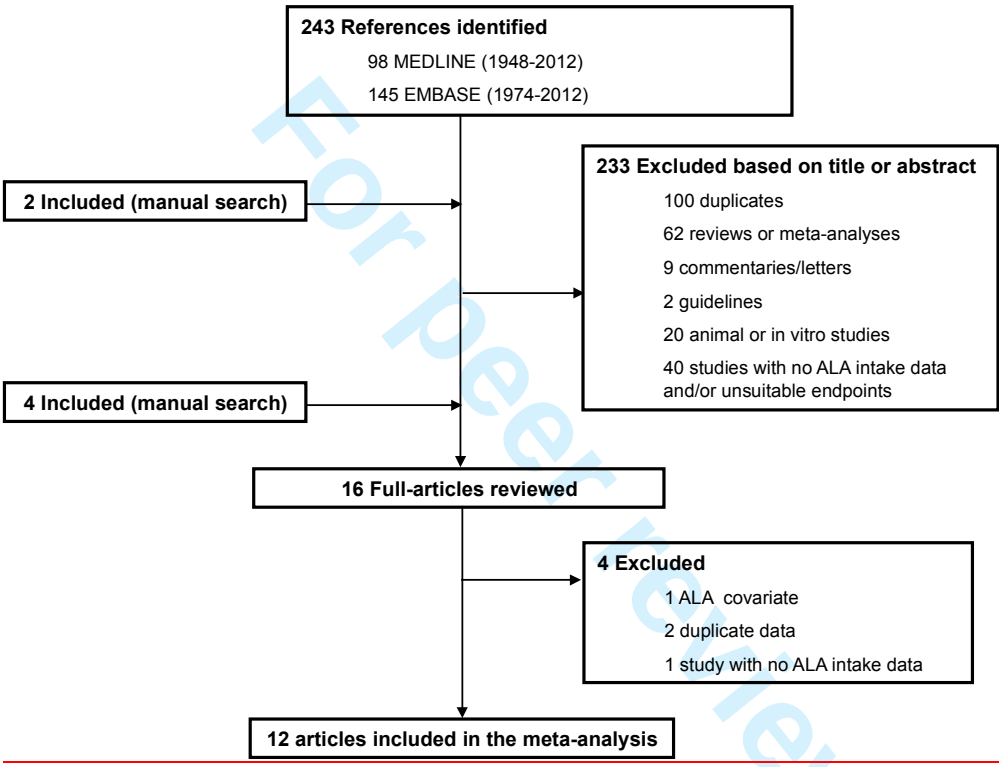

Figure 1 - Flow of the literature.

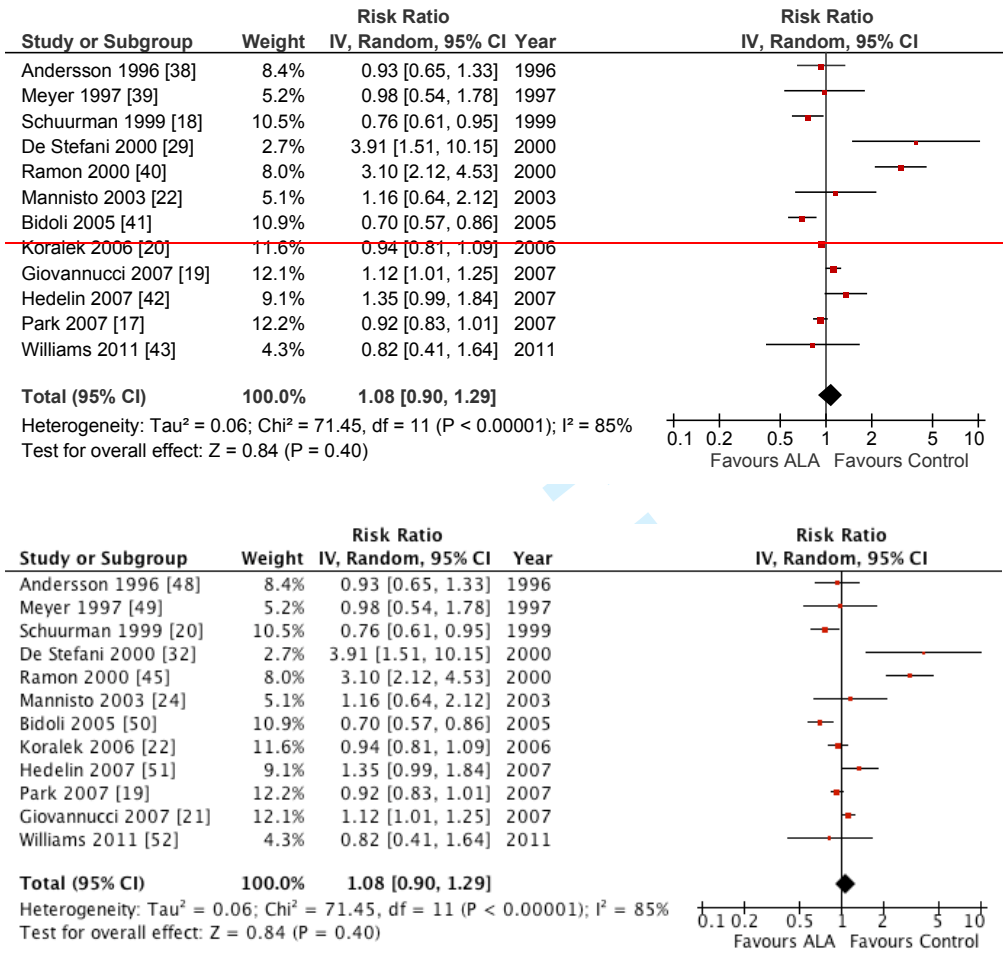

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity[34,55].

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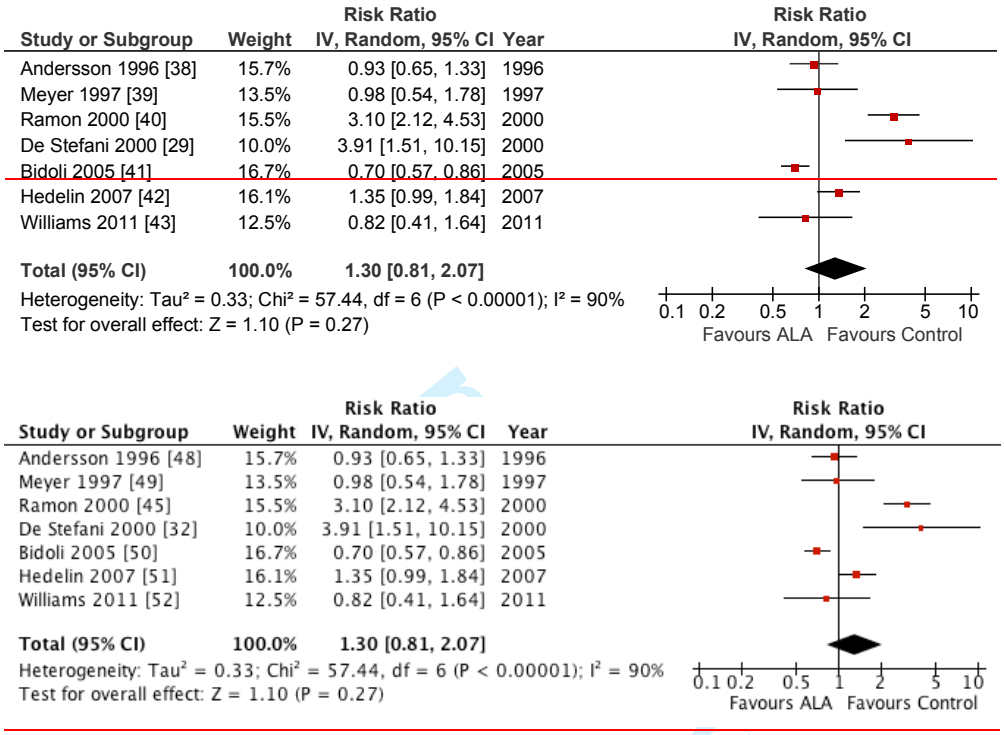

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34,55].

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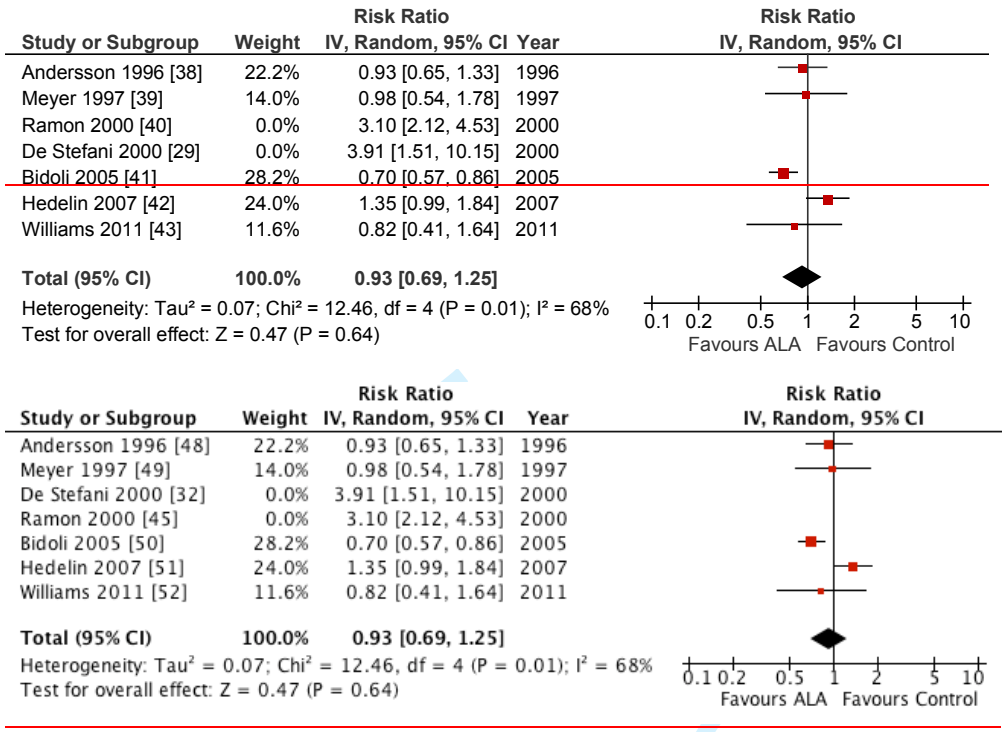

Figure 4 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by ~~Ramon et al.~~ [42] ~~and De Stefani et al.~~De Stefani et al. [32] and Ramon et al. [45] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34][55].

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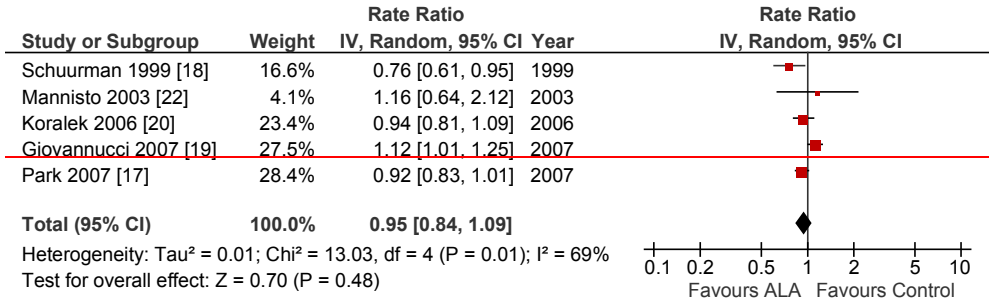
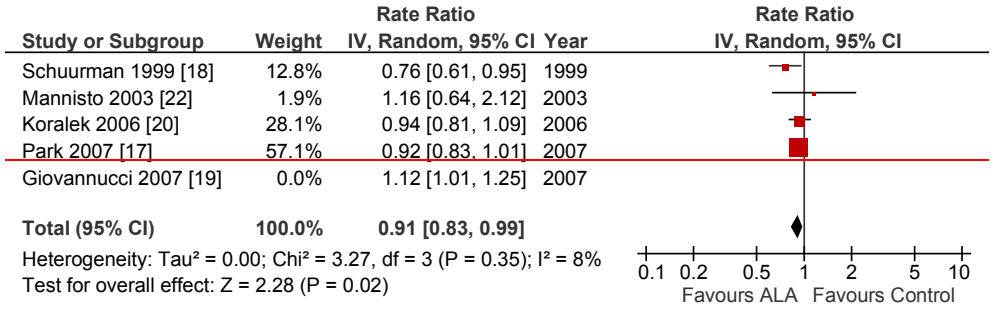


~~**Figure 5** Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34].~~



~~**Figure 6**~~

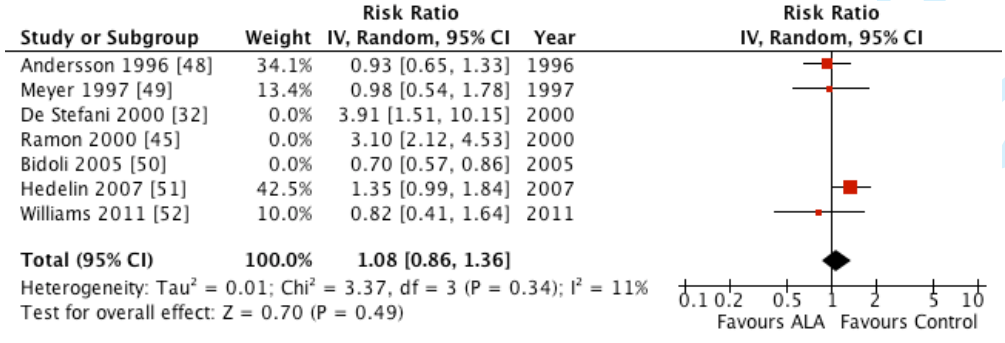


Figure 5 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by De Stefani et al. [32], Ramon et al. [45], and Bidoli et al. [50] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

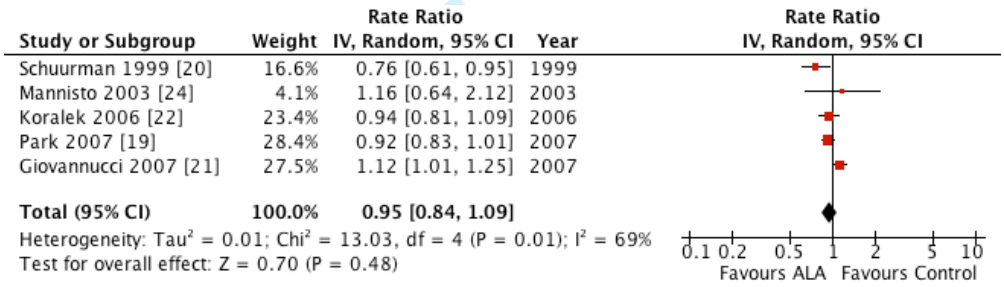


Figure 6 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

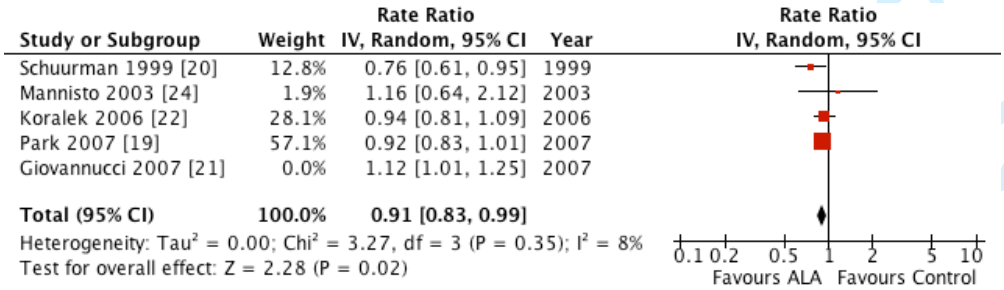


Figure 7 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis.

Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [34][55].

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [26]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.617 - 1.552	0.98	0.65-1.52
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/893 controls	≥65	-	-	-	0.98	0.64-1.76
Schuurman et al. 1999 [16]*	Netherlands	Nested case-cohort	58278 (1535 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.56-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-69	-	-	≥0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2003 [40]	Spain	Case-control	217 cases/434 controls	<80-80	-	-	0.72 - 2.1	3.1	2.3-4.7
Mannisto et al. 2005 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	248	5-8	1.0 - 2.5	1.16	0.84-2.15
Bidoli et al. 2006 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.8	0.7	0.6-0.8
Kolonel et al. 2006 [20]*	United States	Prospective cohort	29,092	65-74	1598	8.1	1.09 - 1.76	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.5	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [18]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.52	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.166†	0.82	0.41-1.65
* Prospective studies.									
† Based on a 2000 kcal diet.									

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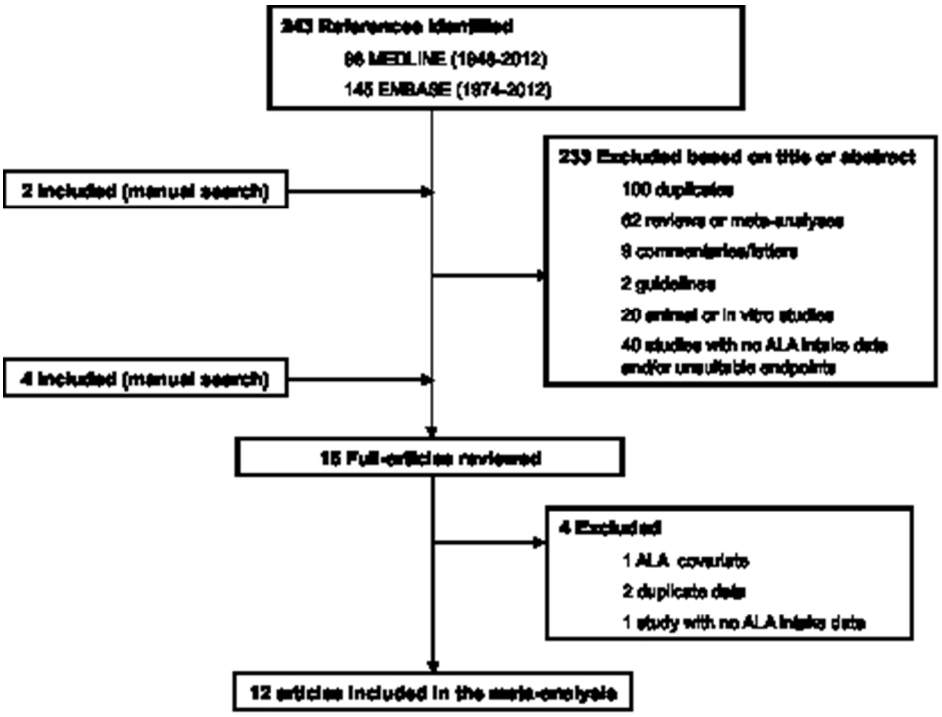

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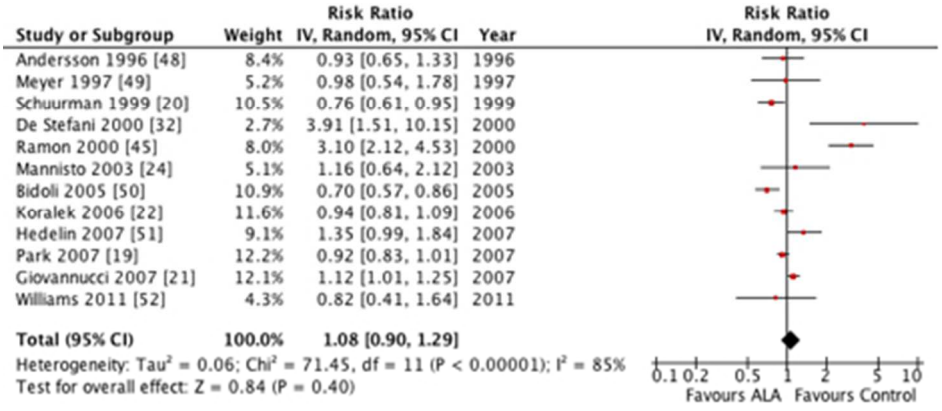

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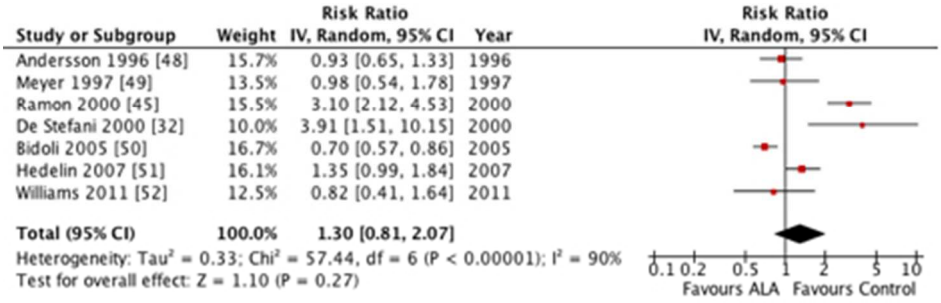

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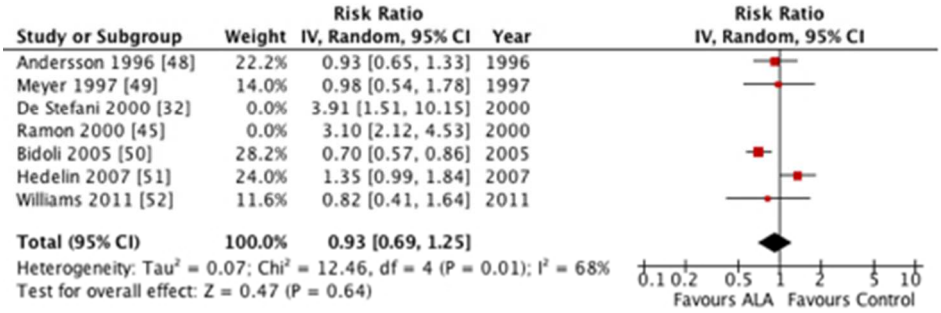

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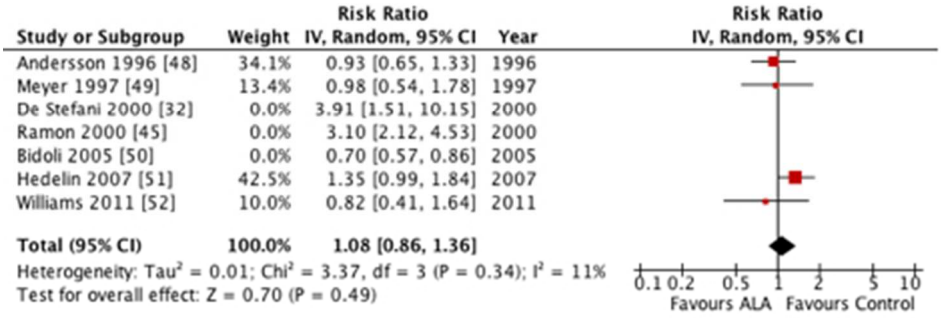

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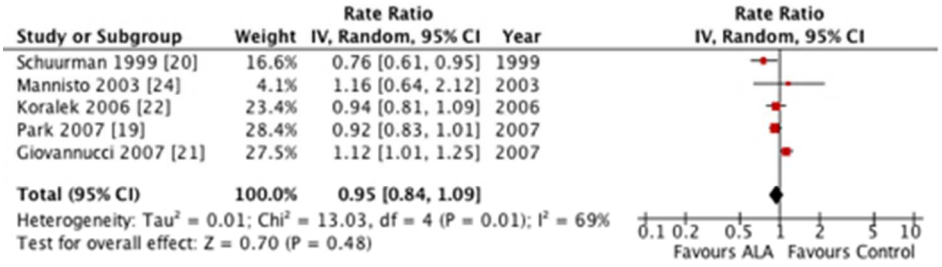

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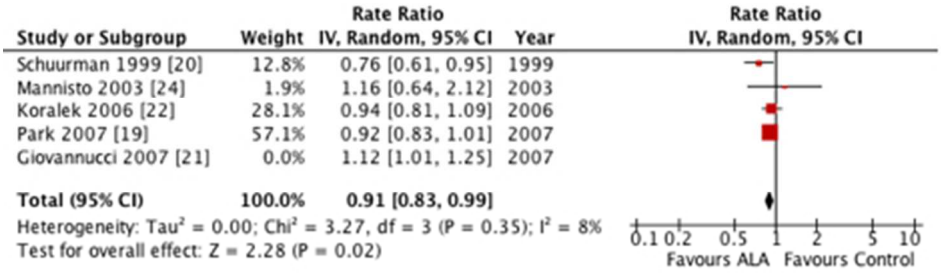

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Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002280.R2
Article Type:	Research
Date Submitted by the Author:	17-Mar-2013
Complete List of Authors:	Carleton, Amanda; University of Toronto, Faculty of Medicine; University of Toronto, Department of Nutritional Sciences Sievenpiper, John; University of Toronto, Department of Nutritional Sciences; St. Michael's Hospital, Risk Factor Modification Centre Jenkins, David; University of Toronto, Department of Nutritional Sciences; St. Michael's Hospital, Risk Factor Modification Centre de Souza, Russell; University of Toronto, Nutritional Sciences McKeown-Eyssen, Gail; University of Toronto, Department of Nutritional Sciences; University of Toronto, Dalla Lana School of Public Health
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Urology, Oncology
Keywords:	NUTRITION & DIETETICS, Prostate disease < UROLOGY, PREVENTIVE MEDICINE

Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

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Text word count: 5300;
Abstract word count: 274;
Tables: 1; Figures: 5;
References: 74

ARTICLE SUMMARY

Article Focus

- ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer
- A systematic review and meta-analysis of case-control and prospective studies was conducted to investigate the association between dietary ALA intake and prostate cancer risk

Key Messages

- The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated overall no significant association between ALA intake and risk of prostate cancer
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies, which reported large odds ratios, the association became non-significantly protective with decreased heterogeneity. The reasons for this result may be explained by the differing sources of ALA
- The subgroup analysis of prospective studies alone showed a protective non-significant association, but with substantial heterogeneity. However, removal of the study by Giovannucci et al. [21] eliminated heterogeneity and the association became significantly protective

Strengths and Limitations:

- This meta-analysis includes both prospective and case control studies to determine the effect of ALA on prostate cancer
- Possible confounders and sources of heterogeneity were discussed and explored in relation to the results
- Interpretation of analyses was complicated by considerable heterogeneity among the studies, which may be due to lack of randomized controlled trials, variation in ALA sources and dietary patterns, variation in ALA exposure levels, differences in FFQs and food databases, variation in adjustment factors, follow-up duration, and study design

ABSTRACT

Background: ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer.

Objective: To conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Data Sources: MEDLINE and EMBASE were searched for relevant prospective and case-control studies.

Eligibility Criteria for Selecting Studies: We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates.

Design: Data were pooled using the generic inverse variance method with a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity was assessed by χ^2 and quantified by I^2.

Results: Data from 5 prospective and 7 case-control studies were pooled. The overall RR estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (1.08 [0.90 to 1.29], P=0.40, I^2=85%), but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies became significant (0.91 [0.83 to 0.99], P=0.02) without evidence of heterogeneity (I^2=8%, P=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

Key Words: Alpha-linolenic acid, prostate cancer, omega-3 fatty acid, meta-analysis

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide [1]. Prostate cancer incidence rates vary widely among countries, populations, and races. Incidence rates vary by more than 25-fold worldwide, with the highest rates documented in the developed countries of North America, Europe, and Oceania, which may be due largely to the wide utilization of prostate- specific antigen (PSA) testing that detects clinically important tumors that might otherwise escape diagnosis [2]. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world [2], which is thought to reflect partly a difference in genetic susceptibility [3 4]. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [5-14]. In 1975, Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [12], and many studies have examined this connection [15-18], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [19-25]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs, since increased consumption of ω-3 fatty acids is advised for cardiovascular disease risk reduction [26-29] despite a possible association with prostate cancer [30].

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [31]. However, in the United States, the important sources of ALA are animal-based foods high in saturated fats, such as red meats, beef, pork, and lamb, rather than ALA-rich vegetable sources, such as walnuts. [25]. The largest proportion of ALA (53.8%) comes from red meat in Uruguay [32], but comes from margarine (25%) in the Netherlands [33]. Furthermore, foods such as bread, eggs, and margarine are now being enriched with ALA to increase their healthfulness.

There are currently divergent health views on ALA. Numerous epidemiological [34-39] and clinical studies [40-42] have shown that ALA is associated with a reduction in coronary heart disease (CHD) incidence and heart disease mortality. However, since ALA has also been associated with an increased risk of prostate cancer, [25 30 32 43-47] the seriousness of this potential

association requires that any favourable effects of ALA on CHD be weighed against its possible adverse effects on prostate cancer. Numerous prospective cohort [19-22 24] and case-control studies [32 45 48-52] have investigated the association between ALA and prostate cancer risk. While previous meta-analyses [30 53 54] have been conducted to determine whether a relationship exists, there has been no meta-analysis since 2010, examining the specific effect of dietary ALA on prostate cancer risk and none since 2009, that included in both prospective cohort and case-control studies. Therefore, it appears timely to determine whether there are associations between dietary ALA from ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.

METHODS

We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [55]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [56].

Study Selection

We first conducted a search of MEDLINE (1948-April 17, 2009) and EMBASE (1974-April 17, 2009) using the following search terms and Boolean operators: *prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids)* and this literature search was last updated on August 28, 2012. The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. We included all prospective cohort, retrospective case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. No lone abstracts or unpublished studies were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information

about study design (prospective cohort, case-control, etc.), sample size and participant characteristics (nationality, race, named cohort, country of residence, gender, age, disease status, preexisting medical conditions), follow-up duration, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors, and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and where necessary by discussion with another investigator (DJAJ). Authors were not contacted to request any additional information or translation.

Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and STATA v. 11.2 (StataCorp, College Station, TX). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. The primary pooled analysis of all reports was conducted using the Generic Inverse Variance method using random effects weighting [57] where the log RRs for cohort studies or log ORs for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer [30 54 58], ORs were considered as approximations of RRs. Since prostate cancer is a rare disease, ORs were treated as unbiased approximations of RRs. [59] Inter-study heterogeneity was assessed by Cochrane's Q (Chi^2 $P<0.10$) and quantified by I^2. An $I^2 \geq 50\%$ indicated "substantial" heterogeneity and $\geq 75\%$ indicated "considerable" heterogeneity. [60] Sources of heterogeneity were explored by sensitivity analyses whereby the influence of individual studies was investigated by systematic removal of each study followed by recalculation of the pooled effect estimate and heterogeneity, as well as removal of outlier studies with risk estimates larger than 2 standard deviations from the mean risk estimate and recalculation of the pooled effect estimate and heterogeneity. We also performed *a priori* subgroup analyses to assess effect modification

by study design (prospective versus case-control). Effect modification by study characteristics was explored using meta-regression . Publication bias was formally tested using Begg's and Egger's tests.

RESULTS

Search Results

Figure 1 shows the flow of the literature selection applying the systematic search and selection strategies to identify eligible reports. Two hundred and forty three reports were identified by the search and two reports were manually included after a database search. Of these, 233 were determined to be irrelevant on review of the titles and abstracts. Four additional reports were then manually included. The remaining 16 reports were retrieved and reviewed in full, of which 4 were excluded. Results for The Health Professionals' Follow-up Study were published in three separate publications at different times of follow-up [21 23 25]. Only the most recent publication of the results, by Giovannucci et al. in 2007, was included in the analyses as representing the cumulative experience of the earlier assessments of this cohort [21]. A total of 12 reports, 5 prospective and 7 case-control studies, were included in the pooled analyses.

Study Characteristics

Table 1 shows the characteristics of the 12 included studies, which were composed of 7 case-control studies [32 45 48-52] and 5 prospective studies [19-22 24] that used 3 designs: cohort, nested case-cohort, and nested case-control. Five studies were conducted in North America, 1 in South America, and 6 in Europe. The 12 included studies contained a total of 14,795 cases of prostate cancer and 231,143 controls. All studies obtained dietary data using food frequency questionnaires (FFQ). Individual and average dietary ALA intake in these studies ranged from ≈0.05 to 4.16 g/d) and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

Primary Analysis

The overall analysis of the 12 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Seven studies reported a protective effect of ALA intake on prostate cancer, one of which was significant, and the remaining five studies reported a

positive association, of which 3 were significant. Overall, high exposure to ALA was not associated with increased risk of prostate cancer (pooled RR: 1.08; 95%CI: 0.90, 1.29, P=0.40) (**Figure 2**). However, there was evidence of considerable inter-study heterogeneity (I^2=85%, P<0.00001). Systematic removal of each study, and recalculation of the pooled effect during sensitivity analyses did not identify an influential outlier.

Subgroup Analyses

Case-Control Studies

In an *a priori* meta-regression, we found no evidence of effect measure modification according to study design (P= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7; 4,047 cases and 4,762 controls), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with considerable inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Systematic removal of each individual study during sensitivity analyses did not explain the heterogeneity. Removal of the 2 case-control studies by Ramon et al.[45], De Stefani et al.[32] that reported risk estimates larger than 2 standard deviations from the pooled RR estimate reduced the inter-study heterogeneity (I^2=68%, P=0.01) but did not eliminate it. The overall association became protective, but was not significant (RR=0.93; 95%CI: 0.69,1.25, P=0.64).

Prospective Studies

In prospective studies alone (n=5; 10,748 cases and 207,752 controls), no association between ALA intake and prostate cancer risk was found (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 4**) but there existed substantial inter-study heterogeneity (I^2=69%, P=0.01). Sensitivity analyses showed that removal of the study by Giovannucci et al. [21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35) and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99, P=0.02) (**Figure 5**).

Publication Bias

Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al. [45] had an unusually large effect with a small standard error.

DISCUSSION

Summary of Results

The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated non-significant heterogeneous effects of ALA on prostate cancer risk. Overall, there was no significant association between ALA intake and risk of prostate cancer. The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies by De Stefani et al. [32] and Ramon et al. [45], which reported large odds ratios greater than 3 but were still within 2 standard deviations of the mean effect, the association became non-significantly protective with decreased heterogeneity. When examining the prospective studies alone, the association between ALA intake and prostate cancer risk was non-significantly protective and after removal of the study by Giovannucci et al. [21] became weakly, but significantly, protective with no heterogeneity.

The results from the prospective studies are similar to those of previously published findings that examined only prospective studies [53]. Our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies and reached the conclusion of non-significantly increased risk with high heterogeneity, particularly due to the inclusion of two studies with very high odds ratios. We explore whether these heterogeneous results can be explained by a number of factors, such as the variation in ALA consumption, sources, or population dietary patterns. However, this heterogeneity among the case-control studies may serve to highlight the less reliable nature of case-control study design as it inherently involves recall bias since dietary information is collected after disease development.

Heterogeneity and the Effect of ALA between Studies

In our study, different findings reviewed and inter-study heterogeneity may be explained by a number of factors: variation in ALA consumption and sources of ALA as a result of the population's dietary patterns, variation in ALA exposure levels, use of different FFQs and food databases, variation in adjustment factors, and difference in follow-up times among prospective studies.

Variation in ALA Consumption and Sources, and Population Dietary Patterns

In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [33], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [25]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [20], but the most recent study from the United States reported a 25% increase in risk [21]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the "healthy" sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels [61 62].

In addition, in the case-control studies from Uruguay [32] and Spain [45] that showed the largest increases in prostate cancer risk demonstrated that meat, and not vegetable, was the major source of ALA. When these two studies were removed from the analysis of the case-control studies, the effect of ALA intake on prostate cancer changed from a non-significantly positive to a non-significantly protective effect. Compared with the other studies from Europe and the United States, there is a much higher consumption of meat in Spain [63] and Uruguay, with Uruguay having the highest meat consumption per capita in the world [64]. An earlier analysis of the Health Professionals Follow-up Study cohort [25] supports this positive association between red meat consumption and prostate cancer risk. Furthermore, the two studies from Spanish-speaking countries also investigated the effect of animal fat on prostate cancer and both found significant positive associations. The Uruguayan study [32] observed that at the highest level of ALA intake derived from animal sources resulted in almost 3 times the risk of developing prostate cancer and the Spanish study [45] revealed that the highest level of animal fat intake was associated with 2 times the risk. These findings indicate that high meat intake rather than high ALA may explain ALA's apparent adverse effect on prostate cancer. In further support of this idea, the study by Bidoli et al.[50] demonstrated a significant protective association between ALA and prostate cancer risk in an Italian population where ALA is mainly derived from olive oil [65] and the diet is rich in raw vegetables [50] rather than meat, profiling an overall more "healthy" diet.

An explanation for the apparent association of prostate cancer incidence with vegetable sources of ALA may be that in addition those who follow healthy lifestyles with increased plant

ALA sources may undergo more frequent prostate specific antigen (PSA) testing and therefore have early prostate cancer detection. In this respect it has been found that higher whole grain intake was also associated with increased prostate cancer risk. However, when frequency of PSA screening was accounted for, the association of whole grains with prostate cancer incidence disappeared [66]. These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk.

Variation in ALA Exposure Levels

Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and/or lower limits of ALA intake [21 32 50] and one study did not report numerical exposure levels [49]. Two studies, one from Spain [45] and one from the Netherlands [20], with the largest adequately defined upper and lower limits of ALA exposure ranges, paradoxically reported the second highest and the second lowest risk of developing prostate cancer, respectively. Since the studies with the greatest range of exposure do not necessarily show the greatest effects, dietary variation in the levels of exposure does not appear to explain differences among the studies, thereby making differences in dietary sources of ALA of more importance especially in relation to meat consumption in Western countries.

Variation in FFQs and Food Databases

In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [67].

Variation in Adjustment Factors

Although all the studies reported adjusted RRs or ORs, the adjustment factors were not consistent among the studies. Some of the adjustment factors in these studies included age,

smoking history, physical activity level, BMI, family history of prostate cancer, history of diabetes mellitus, race, education, socioeconomic status, area of residence and intakes of total calories, fat, processed meat, fish, lycopene, and vitamin E supplements. Currently, the most well-established risk factors for prostate cancer are age, family history of the disease, and race/ethnicity [68] and consequently are the most important adjustment factors. Only 4 [20-22 52] of the 12 included studies adjusted for all of these 3 factors. The studies conducted by Park et al. [19] and Mannisto et al. [24] did not adjust for age, which is by far the strongest predictor of prostate cancer incidence and death [68]. A family history of prostate cancer has been shown to increase the risk of diagnosis and death and this factor was not adjusted for in studies by Hedelin et al. [51], Andersson et al. [48], and Mannisto et al. [24] Race is a prostate cancer risk factor and prognostic factor, with African-American or Black men being at increased risk, and this was not adjusted for in the studies by Bidoli et al. [50], De Stefani et al. [32], Ramon et al. [45], and Meyer et al. [49] Differences in adjustment among the included studies, particularly with respect to the important factors of age, family history of prostate cancer, and race could result in differences in risk estimates, thereby contributing to inter-study heterogeneity.

Variation in Follow-up Duration

Follow-up time may also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from a non-significant to a significant positive association between ALA intake and prostate cancer. So, it can be hypothesized that the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. This association may relate to the development of cancer over a longer period of time and therefore stronger association in the cohort between agents that may cause cancer and tumour occurrence. Alternatively, this relationship may reflect changes in diagnostic effectiveness over time.

Reasons for the Lack of Effect of ALA

The overall effect of ALA on prostate cancer was found to be non-significant but may result from a number of factors including ALA exposure levels that are within health guidelines,

confounding from other polyunsaturated fatty acids, and the difference in effect of ALA on prostate cancer mortality versus incidence.

The mean dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [69], suggesting that ALA may not increase the risk of cancer more than any other nutrient promoting cell growth. Rather, since ALA is a nutrient deficient in the Western diet [70], it may be that a deficiency inhibits all cell growth, including tumour growth, instead of adequate or excess levels causing prostate cancer growth.

Another issue to consider is confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) that might affect ALA metabolism [71] and consequently may introduce bias. The case-control study from the United States [52] demonstrated this as there was no significant association between ALA, omega-3, or omega-6 fatty acids and prostate cancer risk individually, but the highest dietary ratio of omega-6/omega-3 fatty acids was significantly associated with increased risk of high grade prostate cancer.

Finally, our analysis involved cancer incidence rather than mortality and ALA, among other factors such as energy intake, height, body mass index, calcium, and smoking, are also associated with cancer mortality [21]. The study by De Stefani et al. [32], which was the only study that defined cases solely as advanced prostate cancer, had the highest risk estimate of prostate cancer, indicating that ALA may be strongly associated with disease progression rather than incidence. In support of this point, the prospective study by Giovannucci et al. [21] found that higher ALA intake was more strongly associated with increased risk of fatal prostate cancer than with incident. However, three other prospective studies did not find any difference between the effects of ALA on incident or advanced prostate cancer cases [19 20 22]. From these mixed findings, it is unclear whether ALA is associated with severity of prostate cancer, but determining whether ALA impacts prostate cancer incidence or progression is an important distinction that should be investigated in the future. Furthermore, the picture of ALA's effect on prostate cancer is complicated by the positive association of incident prostate cancer with either serum or adipose tissue ALA levels [24 43 44 46 47 72] despite the in vitro evidence which suggests that ALA may suppress prostate cancer cell growth [73 74]. However, there appears to be some correlation between ALA intake and serum ALA levels. In terms of intake, Gann et al. [43] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to

the prospective analysis from the Health Professionals Follow-Up Study [25], they found that red meat was positively associated with advanced prostate cancer, whereas diary foods were not. This corroboration not only suggests a correlation between ALA intake and serum ALA levels, but enforces the positive association between ALA from red meat and prostate cancer as seen in the studies from Uruguay [32] and Spain [45], rather than from plant foods.

Limitations

The first limitation of the meta-analysis is that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Second, interpretation of the analyses was complicated by the evidence of considerable heterogeneity among the studies, which as discussed above may have resulted from differences in ALA sources and population dietary patterns, ALA exposure levels, FFQs and food databases, adjustment factors, and duration of follow-up. There are also inherent limitations in the studies included based on study design. For example, there is the possibility of recall bias in case-control studies, as dietary intake information is collected after disease development.

CONCLUSION

In conclusion, these findings provide no clear evidence of an association between dietary ALA intake and prostate cancer risk. Further, since these observational studies can only show association between ALA intake and prostate cancer, possible causation would be difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, inhibitory, or no effect on prostate cancer risk and development. For the present, no significant association has been found and where any support of a positive effect was seen, red meat sources have been strongly implicated. The source of ALA appears to be of importance, particularly identifying whether it is from animal or vegetable sources, as ALA may be a marker for higher meat and fat intake in some countries both of which have been associated with increased prostate cancer risk. Attention should also be paid to the effect of ALA on prostate cancer progression to address the issues of specific vulnerability identified in the studies of Giovannucci et al. and De Stefani et al. [21 32]. However, resolving the relation of dietary ALA to prostate cancer risk through randomized controlled trials will likely continue to be difficult due to the significant public health implications of

reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Of probably greater importance is determination of the sources of the fatty acid since ALA is associated in the North American diet with meat membranes and creamy salad dressings, which themselves may be markers of a suboptimal dietary pattern and lifestyle

"What this Paper Adds"

ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. Although Carayol et al. conducted a meta-analysis on the effect of dietary ALA on prostate cancer in 2010, only prospective studies were analyzed and case-control studies were not included. Overall, we found no significant association between ALA intake and risk of prostate cancer. The results from the prospective studies were similar to those of previously published findings. However, the subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. The case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain. Additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, null, or inhibitory effect on prostate cancer risk and development.

AUTHORSHIP

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of Contributors: AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS was involved in revising the article critically for important intellectual content. GE was involved

in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

DATA SHARING

There is no additional data available.

COMPETING INTEREST DECLARATION

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) AJC, JLS, RdS, and GE have not had financial support from any company for the submitted work; (2) AJC, JLS, RdS, and GE have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) AJC, JLS, RdS, and GE have no non-financial interests that may be relevant to the submitted work. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla, Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti,

International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet, Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg's, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. DJAJ's wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada.

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58,279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [19]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.32	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.156†	0.82	0.41-1.65

* Prospective studies.

† Based on a 2000 kcal diet.

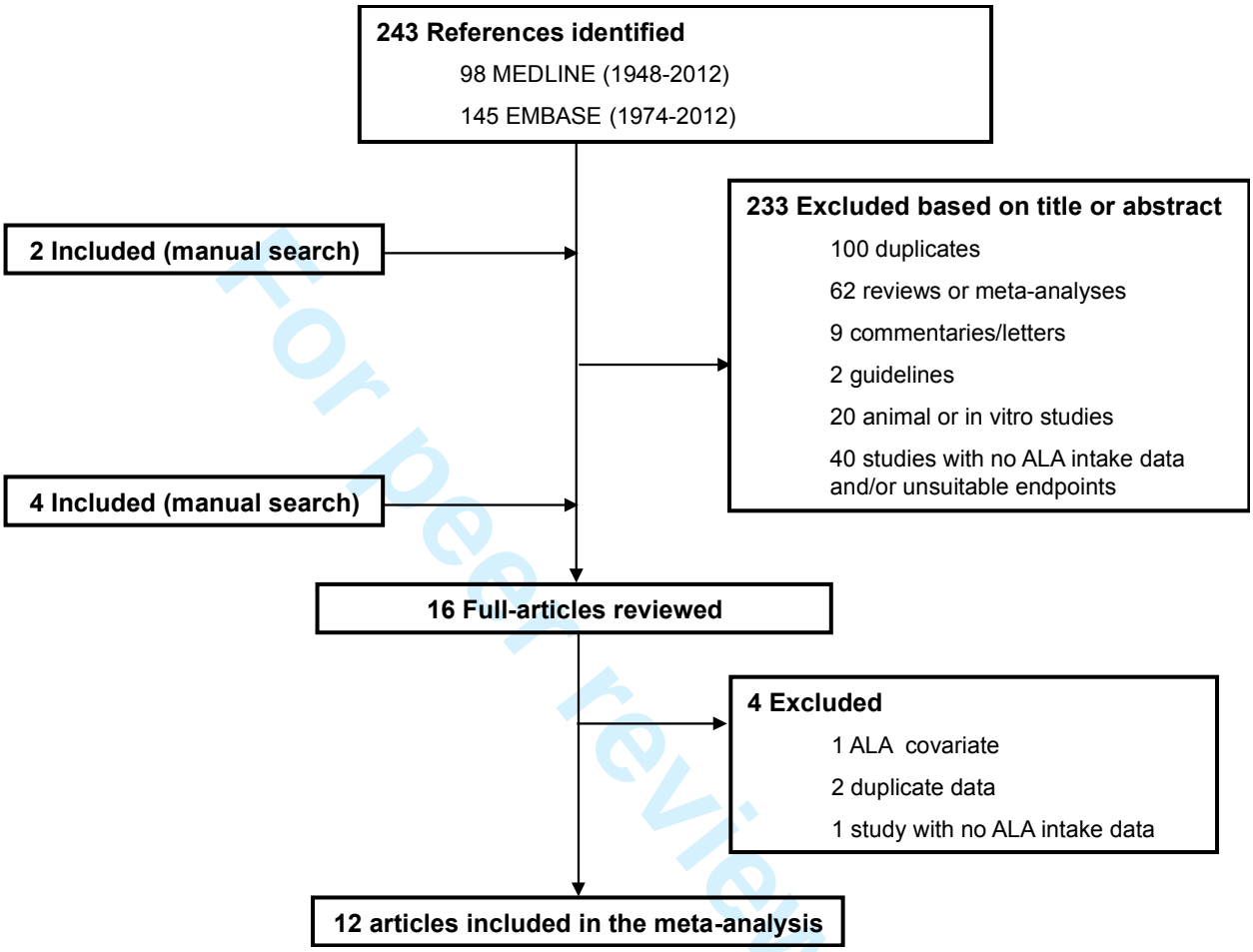

Figure 1 - Flow of the literature.

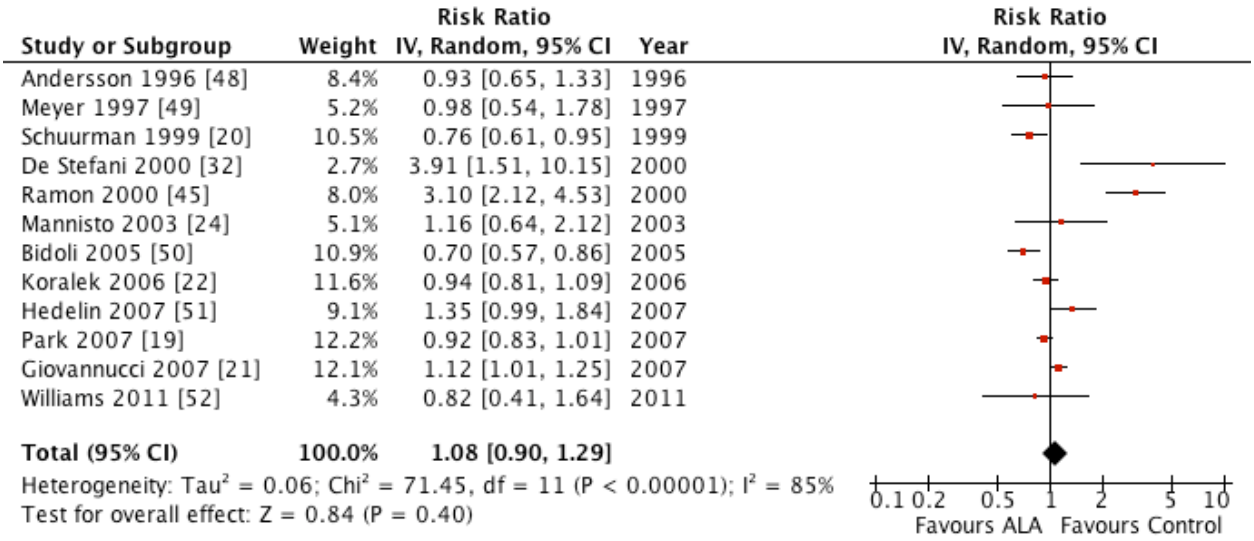

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

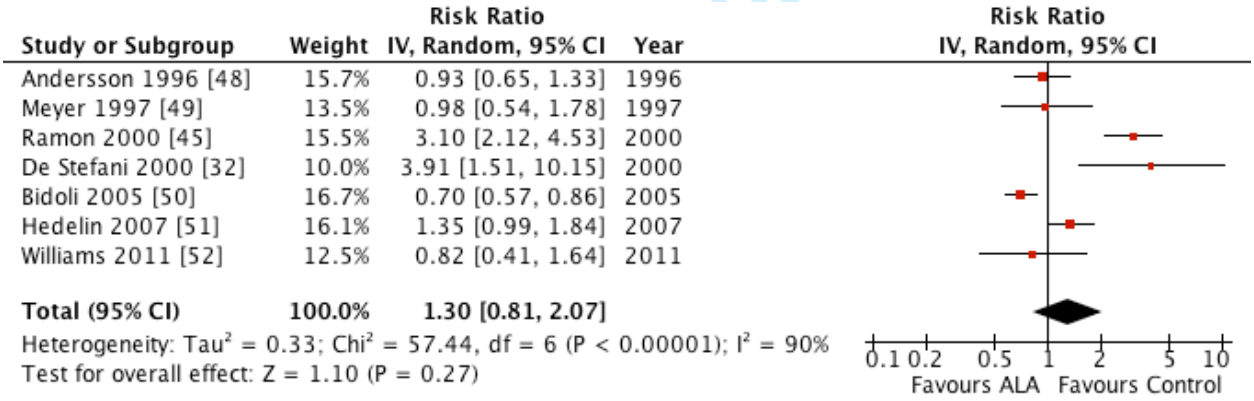

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a

significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

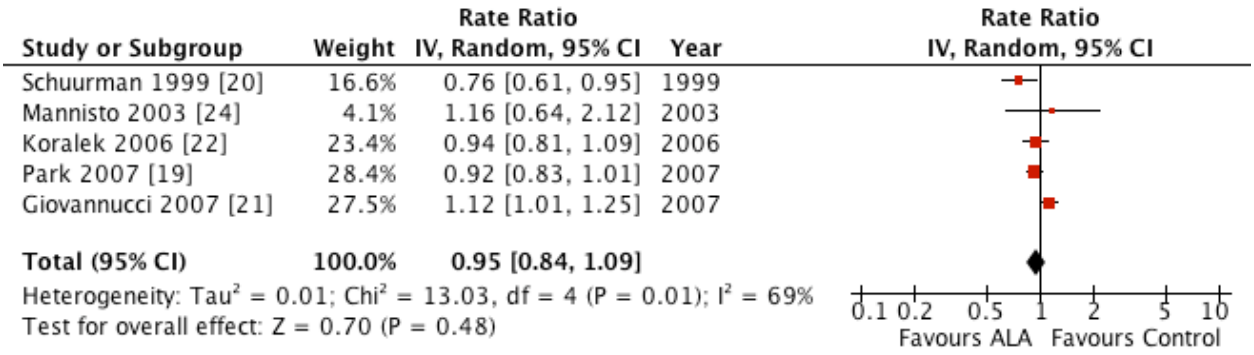

Figure 4 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

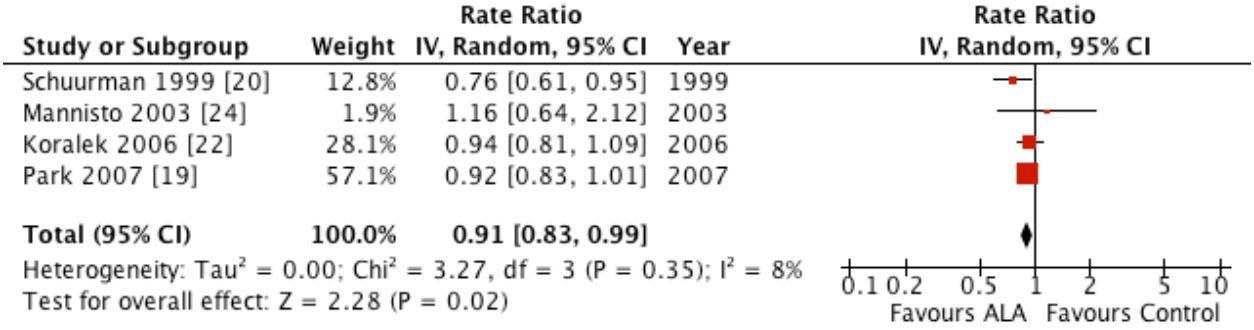

Figure 5 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

Contributorship

AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS was involved in revising the article critically for important intellectual content. GE was involved in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

Funding

None

Competing Interests

None

Data sharing

No additional data available

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Case-Control and Prospective Studies of Dietary Alpha-Linolenic Acid Intake and Prostate Cancer Risk: a Meta-Analysis

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Text word count: 5300~~184~~;
Abstract word count: 274;
Tables: 1; Figures: 5~~7~~;
References: 74

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ABSTRACT

Background: ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer.

Objective: To conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Data Sources: MEDLINE and EMBASE were searched for relevant prospective and case-control studies.

Eligibility Criteria for Selecting Studies: We included all prospective cohort, case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates.

Design: Data were pooled using the generic inverse variance method with a random-effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity was assessed by χ^2 and quantified by I^2.

Results: Data from 5 prospective and 7 case-control studies were pooled. The overall RR estimate showed ALA intake to be positively, but non-significantly associated with prostate cancer risk (1.08 [0.90 to 1.29], P=0.40, I^2=85%), but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies became significant (0.91 [0.83 to 0.99], P=0.02) without evidence of heterogeneity (I^2=8%, P=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

Key Words: Alpha-linolenic acid, prostate cancer, omega-3 fatty acid, meta-analysis

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide [1]. Prostate cancer incidence rates vary widely among countries, populations, and races. Incidence rates vary by more than 25-fold worldwide, with the highest rates documented in the developed countries of North America, Europe, and Oceania, which may be due largely to the wide utilization of prostate- specific antigen (PSA) testing that detects clinically important tumors that might otherwise escape diagnosis [2]. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world [2], which is thought to reflect partly a difference in genetic susceptibility [3 4]. The large differences in prostate cancer incidence rates have led to many migration and ecologic studies, which have provided strong evidence for the role of environmental factors, such as diet, in the etiology of prostate cancer [5-14]. In 1975, Armstrong and Doll first hypothesized that there was an association between dietary fat and death from prostate cancer [12], and many studies have examined this connection [15-18], but in recent years more attention has been focused on specific fatty acids. Several studies have examined the association between polyunsaturated fatty acids (PUFAs) and risk of prostate cancer [19-25]. There has been particular interest in alpha-linolenic acid (ALA), the parent fatty acid for the ω-3 PUFAs, since increased consumption of ω-3 fatty acids is advised for cardiovascular disease risk reduction [26-29] despite a possible association with prostate cancer [30].

Dietary ALA occurs mainly in plants and vegetable oils with certain seed oils (flaxseed, perilla, chia seed, and canola), beans (soybeans, navy beans), and nuts (walnuts) singled out as examples of healthy foods due to their high ALA content [31]. However, in the United States, the important sources of ALA are animal-based foods high in saturated fats, such as red meats, beef, pork, and lamb, rather than ALA-rich vegetable sources, such as walnuts. [25]. The largest proportion of ALA (53.8%) comes from red meat in Uruguay [32], but comes from margarine (25%) in the Netherlands [33]. Furthermore, foods such as bread, eggs, and margarine are now being enriched with ALA to increase their healthfulness.

There are currently divergent health views on ALA. Numerous epidemiological [34-39] and clinical studies [40-42] have shown that ALA is associated with a reduction in coronary heart disease (CHD) incidence and heart disease mortality. However, since ALA has also been associated with an increased risk of prostate cancer, [25 30 32 43-47] the seriousness of this potential

association requires that any favourable effects of ALA on CHD be weighed against its possible adverse effects on prostate cancer. Numerous prospective cohort [19-22 24] and case-control studies [32 45 48-52] have investigated the association between ALA and prostate cancer risk. While previous meta-analyses [30 53 54] have been conducted to determine whether a relationship exists, there has been no meta-analysis since 2010, examining the specific effect of dietary ALA on prostate cancer risk and none since 2009, that included in both prospective cohort and case-control studies. Therefore, it appears timely to determine whether there are associations between dietary ALA from ω-3 fatty acid-rich foods, generally believed to be healthy, and prostate cancer risk.

METHODS

We followed the Cochrane handbook for systematic reviews of interventions version 5.1.0 updated March 2011 for the planning and conduct of this meta-analysis [55]. The reporting followed the QUOROM (Quality of Reporting of Meta-analyses) guidelines [56].

Study Selection

We first conducted a search of MEDLINE (1948-April 17, 2009) and EMBASE (1974-April 17, 2009) using the following search terms and Boolean operators: *prostate AND (cancer OR adenoma OR adenocarcinoma OR neoplasia OR gleason score) AND (alpha-linolenic acid OR n-3 fatty acids OR omega-3 fatty acids)* and this literature search was last updated on August 28, 2012. The search was restricted to human research studies. No limit was placed on language. Manual searches of references cited by the published original studies and review articles supplemented the database search strategy. ~~This search strategy was last updated on August 28, 2012.~~ We included all prospective cohort, retrospective case-control, nested case-cohort, and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), hazard ratios (HR), or odds ratios (OR) estimates. No randomized controlled trials were identified. No lone abstracts or unpublished studies were identified. In cases where multiple publications existed for the same study, the article with the most recent information was included.

Data Extraction

Two investigators (AJC, JLS) independently extracted relevant data on study characteristics and outcomes using a standardized proforma. These data included information

about study design (prospective cohort, case-control, etc.), sample size and participant characteristics (nationality, race, named cohort, country of residence, gender, age, disease status, preexisting medical conditions), follow-up duration, sources of ALA, method of ALA status assessment, endpoints (incidence of prostate cancer, prostate specific antigen (PSA), Gleason score etc.), endpoint assessment (self-reporting, medical records, biopsy, etc.), and number of new incident cases. Bounds of intake categories, quartiles or quintiles, were also recorded. RR, HR, or OR with the greatest degree of control for other environmental and dietary risk factors, and their corresponding 95% CIs for incident prostate cancer risk were extracted as the main endpoint. Disagreements were reconciled by consensus and where necessary by discussion with another investigator (DJAJ). Authors were not contacted to request any additional information or translation.

Statistical Analysis

Data were analyzed using Review Manager (RevMan) 5.1 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and STATA v. 11.2 (StataCorp, College Station, TX). We used the reported RR or OR of the highest versus lowest intake category, as the measure of the relation between ALA intake and prostate cancer risk. ~~A~~ The primary pooled analysis of all reports was conducted using the Generic Inverse Variance method using random effects ~~models~~ weighting [57] where the log RRs for cohort studies or log ORs for case-control studies were weighted by the inverse of the variance to obtain a pooled RR estimate. Since nested case-cohort and nested case-control studies are temporally prospective, we analyzed data from these studies with the prospective studies. As in other meta-analyses that have examined prostate cancer [30 54 58], ORs were considered as approximations of RRs. ~~Since the initial risk of prostate cancer is low~~Since prostate cancer is a rare disease, ~~it is unlikely that there will be a substantial discrepancy in approximating~~ ORs were treated as unbiased approximations of ~~to~~ RRs. [59] Inter-study heterogeneity was assessed by Cochrane's Q (Chi^2 $P<0.10$) and quantified by I^2. An $I^2 \geq 50\%$ indicated "substantial" heterogeneity and $\geq 75\%$ indicated "considerable" heterogeneity. [60] Sources of heterogeneity were explored by sensitivity analyses whereby the influence of individual studies was investigated by systematic removal of each study followed by recalculation of the pooled effect estimate and heterogeneity, as well as removal of outlier studies with risk estimates larger than 2 standard deviations from the mean risk estimate and

recalculation of the pooled effect estimate and heterogeneity. We also performed *a priori* subgroup analyses to assess effect modification by study design (prospective versus case-control). Effect modification by study characteristics was explored using meta-regression ~~Post-hoc analyses included dichotomous subgroup analyses to assess effect modification by study design (STATA 11.2., College Station, USA) and continuous analyses to assess the effect of the duration of follow-up on relative risk among prospective studies~~. Publication bias ~~that~~ was formally tested using Begg's and Egger's tests.

RESULTS

Search Results

Figure 1 shows the flow of the literature selection applying the systematic search and selection strategies to identify eligible reports. Two hundred and forty three reports were identified by the search and two reports were manually included after a database search. Of these, 233 were determined to be irrelevant on review of the titles and abstracts. Four additional reports were then manually included. The remaining 16 reports were retrieved and reviewed in full, of which 4 were excluded. Results for The Health Professionals' Follow-up Study were published in three separate publications at different times of follow-up [21 23 25]. Only the most recent publication of the results, by Giovannucci et al. in 2007, was included in the analyses as representing the cumulative experience of the earlier assessments of this cohort [21]. A total of 12 reports, 5 prospective and 7 case-control studies, were included in the pooled analyses.

Study Characteristics

Table 1 shows the characteristics of the 12 included studies, which were composed of 7 case-control studies [32 45 48-52] and 5 prospective studies [19-22 24] that used 3 designs: cohort, nested case-cohort, and nested case-control. Five studies were conducted in North America, 1 in South America, and 6 in Europe. The 12 included studies contained a total of 14,795 cases of prostate cancer and 231,143 controls. All studies obtained dietary data using food frequency questionnaires (FFQ). Individual and average dietary ALA intake in these studies ranged from ≈0.05 to 4.16 g/d) and the reported relative risk or odds ratio of the highest versus the lowest intake category ranged from 0.7 to 3.91.

Primary Analysis

The overall analysis of the 12 studies examined prostate cancer, comparing the highest with the lowest ALA intake category. Seven studies reported a protective effect of ALA intake on prostate cancer, one of which was significant, and the remaining five studies reported a positive association, of which 3 were significant. ~~Overall, although the relative risk was increased numerically by 8%,~~ Overall, high exposure to ALA was not associated with increased risk of prostate cancer ~~this increase in prostate cancer risk was not significant~~ (pooled RR: 1.08; 95%CI: 0.90, 1.29, P=0.40) (**Figure 2**). However, there was evidence of considerable inter-study heterogeneity (I^2=85%, P<0.00001). Systematic removal of each study, and recalculation of the pooled effect~~-~~ during sensitivity analyses did not ~~suggest~~ identify ~~any single study was~~ an influential outlier.

Subgroup Analyses

Case-Control Studies

In an *a priori* meta-regression, we found no evidence of effect measure modification according to study design (~~P-value of the associated beta coefficient for study design~~ P for ~~heterogeneity~~= 0.331). There remained significant unexplained heterogeneity within each type of study design. In case-control studies (n=7; 4,047 ~~n~~ cases and 4,762~~n~~ controls), the summary RR was 1.30 (95%CI: 0.81, 2.07, P=0.27), with considerable inter-study heterogeneity (I^2=90%, P<0.00001) (**Figure 3**). Systematic removal of each individual study during sensitivity analyses did not explain the heterogeneity. Removal of the 2 case-control studies by Ramon et al.[45], De Stefani et al.[32] that reported risk estimates larger than 2 standard deviations from the pooled RR estimate reduced the inter-study heterogeneity (I^2=68%, P=0.01) but did not eliminate it ~~(**Figure 4**)~~. The overall association became ~~weakly~~ protective, but was not significant (RR=0.93; 95%CI: 0.69,1.25, P=0.64) ~~(**Figure 4**)~~. ~~Removal of the 3 case-control studies by Ramon et al.[45], De Stefani et al.[32], and Bidoli et al.,[50] that had risk estimates outside the 95% CI of the pooled RR estimate, eliminated heterogeneity in the case-control studies (I^2=11%, P=0.34), but the overall non-significant association between ALA intake and prostate cancer risk remained (RR=1.08; 95%CI: 0.86,1.36, P=0.49) (**Figure 5**).~~

Prospective Studies

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In prospective studies alone (n=5; 10,748~~n~~ cases and 207,752~~n~~ controls), no association between ALA intake and prostate cancer risk was ~~revealed~~ found (RR: 0.95; 95%CI: 0.84, 1.09, P=0.48) (**Figure 46**) but there existed substantial inter-study heterogeneity (I^2=69%, P=0.01). Sensitivity analyses showed that removal of the study by Giovannucci et al. [21] eliminated heterogeneity with prospective studies (I^2=8%, P=0.35) and made the protective effect significant (RR=0.91; 95%CI: 0.83,0.99, P=0.02) (**Figure 57**).

~~Duration of follow-up in prospective studies was found to be positively but not significantly associated with the magnitude of relative risk (r=0.47).~~

Publication Bias

Neither Begg's (P>0.165) nor Egger's (P>0.527) tests revealed evidence of publication bias, however, one study by Ramon et al. [45] had an unusually large effect with a small standard error.

DISCUSSION

Summary of Results

The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated non-significant heterogeneous effects of ALA on prostate cancer risk. Overall, there was no significant association between ALA intake and risk of prostate cancer. The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies by De Stefani et al. [32] and Ramon et al. [45], which reported large odds ratios greater than 3 but were still within 2 standard deviations of the mean effect, the association became ~~weakly~~ non-significantly protective with decreased heterogeneity. When examining the prospective studies alone, the association between ALA intake and prostate cancer risk was ~~weakly~~ non-significantly protective and after removal of the study by Giovannucci et al. [21] became weakly, but significantly, protective with no heterogeneity.

The results from the prospective studies are similar to those of previously published findings that examined only prospective studies [53]. Our study additionally investigated the association between dietary ALA intake and prostate cancer risk among case-control studies and

Comment [R1]: I would not place too much emphasis on this... the magnitude of change is small (from 0.95 to 0.91), and the CI's both with and without Giovannucci both include each estimate. So yes, it's nice that we got "significance", but let's not take this as a strong finding, unless there's a strong reason why Ed got it wrong.

reached ~~a similar~~the conclusion of non-significantly increased risk with high heterogeneity, particularly due to the inclusion of two studies with very high odds ratios. We explore whether these heterogeneous results can be explained by a number of factors, such as the variation in ALA consumption, sources, or population dietary patterns. However, this heterogeneity among the case-control studies may serve to highlight the less reliable nature of case-control study design as it inherently involves recall bias since dietary information is collected after disease development.

~~although the case-control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain.~~

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Heterogeneity and the Effect of ALA between Studies

In our study, different findings reviewed and inter-study heterogeneity may be explained by a number of factors: variation in ALA consumption and sources of ALA as a result of the population's dietary patterns, variation in ALA exposure levels, use of different FFQs and food databases, variation in adjustment factors, and difference in follow-up times among prospective studies.

Variation in ALA Consumption and Sources, and Population Dietary Patterns~~.~~

In the Netherlands, the chief sources of ALA include margarine (25% of daily intake), meat (11%), bread (10%), and vegetables (8%) [33], whereas in the United States, major sources of ALA come from mayonnaise, creamy salad dressings, margarine, butter, beef, pork, lamb, and oil and vinegar-based dressings [25]. Interestingly, the prospective study from the Netherlands reported a weak protective effect of ALA intake on prostate cancer risk [20], but the most recent study from the United States reported a 25% increase in risk [21]. This difference may be due to the nature of the foods that contain ALA since in the United States, the sources of ALA are not the "healthy" sources where ALA is naturally found (e.g. flaxseed, walnuts, and canola oil), but rather profiled an unhealthy diet (e.g. canola oil in the form of mayonnaise and creamy salad dressings), which may be indicative of a less healthy lifestyle and this in itself may contribute to an increased risk of prostate cancer independent of ALA intake levels [61 62].

In addition, in the case-control studies from Uruguay [32] and Spain [45] that showed the largest increases in prostate cancer risk demonstrated that meat, and not vegetable, was the major source of ALA. When these two studies were removed from the analysis of the case-control studies, the effect of ALA intake on prostate cancer changed from a non-significantly ~~weakly~~ positive to a non-significantly~~weakly~~ protective effect. Compared with the other studies from Europe and the United States, there is a much higher consumption of meat in Spain [63] and Uruguay, with Uruguay having the highest meat consumption per capita in the world [64]. An earlier analysis of the Health Professionals Follow-up Study cohort [25] supports this positive association between red meat consumption and prostate cancer risk. Furthermore, the two studies from Spanish-speaking countries also investigated the effect of animal fat on prostate cancer and both found significant positive associations. The Uruguayan study [32] observed that at the highest level of ALA intake derived from animal sources resulted in almost 3 times the risk of developing prostate cancer and the Spanish study [45] revealed that the highest level of animal fat intake was associated with 2 times the risk. These findings indicate that high meat intake rather than high ALA may explain ALA's apparent adverse effect on prostate cancer. In further support of this idea, the study by Bidoli et al.[50] demonstrated a significant protective association between ALA and prostate cancer risk in an Italian population where ALA is mainly derived from olive oil [65] and the diet is rich in raw vegetables [50] rather than meat, profiling an overall more "healthy" diet.

An explanation for the apparent association of prostate cancer incidence with vegetable sources of ALA may be that in addition those who follow healthy lifestyles with increased plant ALA sources may undergo more frequent prostate specific antigen (PSA) testing and therefore have early prostate cancer detection. In this respect it has been found that higher whole grain intake was also associated with increased prostate cancer risk. However, when frequency of PSA screening was accounted for, the association of whole grains with prostate cancer incidence disappeared [66]. These studies indicate the importance of not only identifying the dietary sources of ALA, but taking into account what the nature of the foods may indicate in terms of diet and lifestyle since these also may affect prostate cancer risk.

Variation in ALA Exposure Levels~~.~~

Another important aspect to consider is the differing exposure levels between the studies. Each study had different cut-offs for each quantile, which makes a true comparison of ALA intake exposure difficult since some studies had higher levels of ALA in their highest intake quantile than others. Further, some studies did not adequately define the absolute upper and/or lower limits of ALA intake [21 32 50] and one study did not report numerical exposure levels [49]. Two studies, one from Spain [45] and one from the Netherlands [20], with the largest adequately defined upper and lower limits of ALA exposure ranges, paradoxically reported the second highest and the second lowest risk of developing prostate cancer, respectively. Since the studies with the greatest range of exposure do not necessarily show the greatest effects, dietary variation in the levels of exposure does not appear to explain differences among the studies, thereby making differences in dietary sources of ALA of more importance especially in relation to meat consumption in Western countries.

Variation in FFQs and Food Databases

In terms of utilizing different FFQs and food databases, each study used a different dietary FFQ. ALA content of processed food can vary, which can be of concern when using food databases to translate food intake into fatty acid intake. For example, the ALA content of 12 margarines available in Australia range from 0.2% to 5.9% [67].

Variation in Adjustment Factors

Although all the studies reported adjusted RRs or ORs, the adjustment factors were not consistent among the studies. Some of the adjustment factors in these studies included age, smoking history, physical activity level, BMI, family history of prostate cancer, history of diabetes mellitus, race, education, socioeconomic status, area of residence and intakes of total calories, fat, processed meat, fish, lycopene, and vitamin E supplements. Currently, the most well-established risk factors for prostate cancer are age, family history of the disease, and race/ethnicity [68] and consequently are the most important adjustment factors. Only 4 [20-22 52] of the 12 included studies adjusted for all of these 3 factors. The studies conducted by Park et al. [19] and Mannisto et al. [24] did not adjust for age, which is by far the strongest predictor of prostate cancer incidence and death [68]. A family history of prostate cancer has been shown to increase the risk of diagnosis and death and this factor was not adjusted for in studies by Hedelin et al. [51], Andersson

et al. [48], and Mannisto et al. [24] Race is a prostate cancer risk factor and prognostic factor, with African-American or Black men being at increased risk, and this was not adjusted for in the studies by Bidoli et al. [50], De Stefani et al. [32], Ramon et al. [45], and Meyer et al. [49] Differences in adjustment among the included studies, particularly with respect to the important factors of age, family history of prostate cancer, and race could result in differences in risk estimates, thereby contributing to inter-study heterogeneity.

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Variation in Follow-up Duration

Follow-up time may also have an effect on heterogeneity, especially since the study by Giovannucci et al. [21] had the longest follow-up duration (16 years). Comparing previous prospective studies following the same cohort [23 25] with this most recent study [21], demonstrates a shift over time (total of 12 years) from a non-significant to a significant positive association between ALA intake and prostate cancer. So, it can be hypothesized that the heterogeneity induced by this study may indicate that follow-up duration is positively related to the strength of the association between ALA and prostate cancer risk. This association may relate to the development of cancer over a longer period of time and therefore stronger association in the cohort between agents that may cause cancer and tumour occurrence. Alternatively, this relationship may reflect changes in diagnostic effectiveness over time. ~~After investigating this suggestion, the effect of follow-up duration on relative risk among the prospective studies was found to be positively, but not significantly correlated (r=0.47).~~

Reasons for the Lack of Effect of ALA

The overall effect of ALA on prostate cancer was found to be non-significant but may result from a number of factors including ALA exposure levels that are within health guidelines, confounding from other polyunsaturated fatty acids, and the difference in effect of ALA on prostate cancer mortality versus incidence.

The mean dietary ALA intake levels observed in these studies were all within the dietary reference intake (DRI) range of 1.1 to 1.6 g/d [69], suggesting that ALA may not increase the risk of cancer more than any other nutrient promoting cell growth. Rather, since ALA is a nutrient deficient in the Western diet [70], it may be that a deficiency inhibits all cell growth, including tumour growth, instead of adequate or excess levels causing prostate cancer growth.

Another issue to consider is confounding from other polyunsaturated fatty acids such as omega-6 or other omega-3 fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) that might affect ALA metabolism [71] and consequently may introduce bias. The case-control study from the United States [52] demonstrated this as there was no significant association between ALA, omega-3, or omega-6 fatty acids and prostate cancer risk individually, but the highest dietary ratio of omega-6/omega-3 fatty acids was significantly associated with increased risk of high grade prostate cancer.

Finally, our analysis involved cancer incidence rather than mortality and ALA, among other factors such as energy intake, height, body mass index, calcium, and smoking, are also associated with cancer mortality [21]. The study by De Stefani et al. [32], which was the only study that defined cases solely as advanced prostate cancer, had the highest risk estimate of prostate cancer, indicating that ALA may be strongly associated with disease ~~severity~~ progression rather than incidence. In support of this point, the prospective study by Giovannucci et al. [21] found that higher ALA intake was more strongly associated with increased risk of fatal prostate cancer than with incident. However, three other prospective studies did not find any difference between the effects of ALA on incident or advanced prostate cancer cases [19 20 22]. From these mixed findings, it is unclear whether ALA is associated with severity of prostate cancer, but determining whether ALA impacts prostate cancer incidence or progression is an important distinction that should be investigated in the future. Furthermore, the picture of ALA's effect on prostate cancer is complicated by the positive association of incident prostate cancer with either serum or adipose tissue ALA levels [24 43 44 46 47 72] despite the in vitro evidence which suggests that ALA may suppress prostate cancer cell growth [73 74]. However, there appears to be some correlation between ALA intake and serum ALA levels. In terms of intake, Gann et al. [43] found that plasma ALA levels were significantly positively correlated with meat and dairy product intake, and similar to the prospective analysis from the Health Professionals Follow-Up Study [25], they found that red meat was positively associated with advanced prostate cancer, whereas diary foods were not. This corroboration not only suggests a correlation between ALA intake and serum ALA levels, but enforces the positive association between ALA from red meat and prostate cancer as seen in the studies from Uruguay [32] and Spain [45], rather than from plant foods.

Limitations

The first limitation of the meta-analysis is that all data currently available for inclusion come from epidemiological studies since there are no data from randomized controlled trials due to ethical concerns. Second, interpretation of the analyses was complicated by the evidence of considerable heterogeneity among the studies, which as discussed above may have resulted from differences in ALA sources and population dietary patterns, ALA exposure levels, FFQs and food databases, adjustment factors, and duration of follow-up. There are also inherent limitations in the studies included based on study design. ~~The association between ALA intake and prostate cancer risk was stronger overall in the case-control studies than in the prospective studies. However~~For example, there is the possibility of recall bias in case-control studies, as dietary intake information is collected after disease development.

CONCLUSION

In conclusion, these findings provide no clear evidence of an association between dietary ALA intake and prostate cancer risk. Further, since these observational studies can only show association between ALA intake and prostate cancer, possible causation would be difficult to establish. Therefore, additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, inhibitory, or no effect on prostate cancer risk and development. For the present, no significant association has been found and where any support of a positive effect was seen, red meat sources have been strongly implicated. The source of ALA appears to be of importance, particularly identifying whether it is from animal or vegetable sources, as ALA may be a marker for higher meat and fat intake in some countries both of which have been associated with increased prostate cancer risk. Attention should also be paid to the effect of ALA on prostate cancer progression to address the issues of specific vulnerability identified in the studies of Giovannucci et al. and De Stefani et al. [21 32]. However, resolving the relation of dietary ALA to prostate cancer risk through randomized controlled trials will likely continue to be difficult due to the significant public health implications of reducing/eliminating a dietary fatty acid which is essential and has suggested heart health benefits. Of probably greater importance is determination of the sources of the fatty acid since ALA is associated in the North American diet with meat membranes and creamy salad dressings, which themselves may be markers of a suboptimal dietary pattern and lifestyle

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ARTICLE SUMMARY

Article Focus

- ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer
- A systematic review and meta-analysis of case-control and prospective studies was conducted to investigate the association between dietary ALA intake and prostate cancer risk

Key M~~m~~essages

- The present meta-analysis of 12 observational studies (7 case-control and 5 prospective) comparing the highest with the lowest categories of dietary ALA intake demonstrated overall no significant association between ALA intake and risk of prostate cancer
- The subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. However, upon removal of the studies, which reported large odds ratios, the association became ~~weakly~~ non-significantly protective ~~but remained non-significant,~~ with decreased heterogeneity. The reasons for this result may be explained by the differing sources of ALA
- The subgroup analysis of prospective studies alone showed a protective non-significant association, but with substantial heterogeneity. However, removal of the study by Giovannucci et al. [21] eliminated heterogeneity and the association became significantly protective~~case control studies alone showed a positive non-significant association, but with substantial heterogeneity,~~
- ~~which suggests an element of increased risk dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain~~

Strengths and Limitations:

- This meta-analysis includes both prospective and case control studies to determine the effect of ALA on prostate cancer
- Possible confounders and sources of heterogeneity were discussed and explored in relation to the results
- Interpretation of analyses was complicated by considerable heterogeneity among the studies, which may be due to lack of randomized controlled trials, variation in ALA

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sources and dietary patterns, variation in ALA exposure levels, differences in FFQs and food databases, variation in adjustment factors, follow-up duration, and study design

"What this Paper Adds"

ALA is considered a cardioprotective nutrient, however some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. Although Carayol et al. conducted a meta-analysis on the effect of dietary ALA on prostate cancer in 2010, only prospective studies were analyzed and case-control studies were not included. Overall, we found no significant association between ALA intake and risk of prostate cancer. The results from the prospective studies were similar to those of previously published findings. However, the subgroup analysis of case control studies alone showed a positive non-significant association, but with substantial heterogeneity. The case control studies suggested an element of increased risk, which was dependent on the inclusion of two studies with very high odds ratios, the reasons for which are difficult to explain. Additional research from epidemiological, clinical, and in vitro studies are required to elucidate whether ALA has a promotional, null, or inhibitory effect on prostate cancer risk and development.

AUTHORSHIP

All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of Contributors: AJC was involved in the conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be published. JLS was involved in the conception and design, some analysis, and revising the article critically for important intellectual content. RS was involved in revising the article critically for important intellectual content. GE was involved in the conception and design and in revising the article critically for important intellectual content. DJAJ was in the conception and design, revising the article critically for important intellectual content, and final approval of the version to be published.

DATA SHARING

There is no additional data available.

COMPETING INTEREST DECLARATION

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) AJC, JLS, RdS, and GE have not had financial support from any company for the submitted work; (2) AJC, JLS, RdS, and GE have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) AJC, JLS, RdS, and GE have no non-financial interests that may be relevant to the submitted work. DJAJ has served on the Scientific Advisory Board of Sanitarium Company, Agri-Culture and Agri-Food Canada (AAFC), Canadian Agriculture Policy Institute (CAPI), California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; received honoraria for scientific advice from Sanitarium Company, Orafti, the Almond Board of California, the American Peanut Council, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, Herbal Life International, Pacific Health Laboratories, Nutritional Fundamental for Health, Barilla, Metagenics, Bayer Consumer Care, Unilever Canada and Netherlands, Solae, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, Pepsi, and Alpro Foundation; has been on the speakers panel for the Almond Board of California; received research grants from Saskatchewan Pulse Growers, the Agricultural Bioproducts Innovation Program (ABIP) through the Pulse Research Network (PURENet), Advanced Food Materials Network (AFMNet), Loblaw, Unilever, Barilla, Almond Board of California, Coca-Cola, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council Nutrition Research and Education Foundation and the Peanut Institute, the Canola and Flax Councils of Canada, Calorie Control Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund; and received travel support to meetings from the Solae, Sanitarium Company, Orafti, AFMNet,

Comment [R2]: May want to confirm John is okay with this—his wife works for Unilever. He and I generally disclose our Coke ties, but might not be relevant for this—we like to err on the side of "overidsclosure"

Coca-Cola, The Canola and Flax Councils of Canada, Oldways Preservation Trust, Kellogg's, Quaker Oats, Griffin Hospital, Abbott Laboratories, Dean Foods, the California Strawberry Commission, American Peanut Council, Herbal Life International, Nutritional Fundamental for Health, Metagenics, Bayer Consumer Care, AAFC, CAPI, Pepsi, Almond Board of California, Unilever, Alpro Foundation, International Tree Nut Council, Barilla, Pulse Canada, and the Saskatchewan Pulse Growers. DJAJ's wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada.

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Exposure level (g/d)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [38]	Sweden	Case-control	526 cases/536 controls	<80	-	-	0.817 - 1.352	0.93	0.65-1.32
Meyer et al. 1997 [39]	Canada	Case-control	215 cases/593 controls	≥45	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [18]*	Netherlands	Nested case-cohort	58,279 (1525 subcohort)	55-69	642	6.3	0.7 - 2.1	0.76	0.66-1.04
De Stefani et al. 2000 [29]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.50-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<60-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	246	5-8	1.0 - 2.3	1.16	0.64-2.13
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	45-74	-	-	mean 1.6	0.7	0.6-0.9
Koralek et al. 2006 [20]*	United States	Prospective cohort	29,592	55-74	1898	5.1	1.09 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.3	-	-	0.05 - 0.60	1.35	0.99-1.84
Giovannucci et al. 2007 [19]*	United States	Prospective cohort	47,750	40-75	3544	16	<0.79 - ≥1.32	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,483	≥45	4404	8	1.1 - 2.14†	0.92	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.0 - 4.156†	0.82	0.41-1.65

* Prospective studies.
† Based on a 2000 kcal diet.

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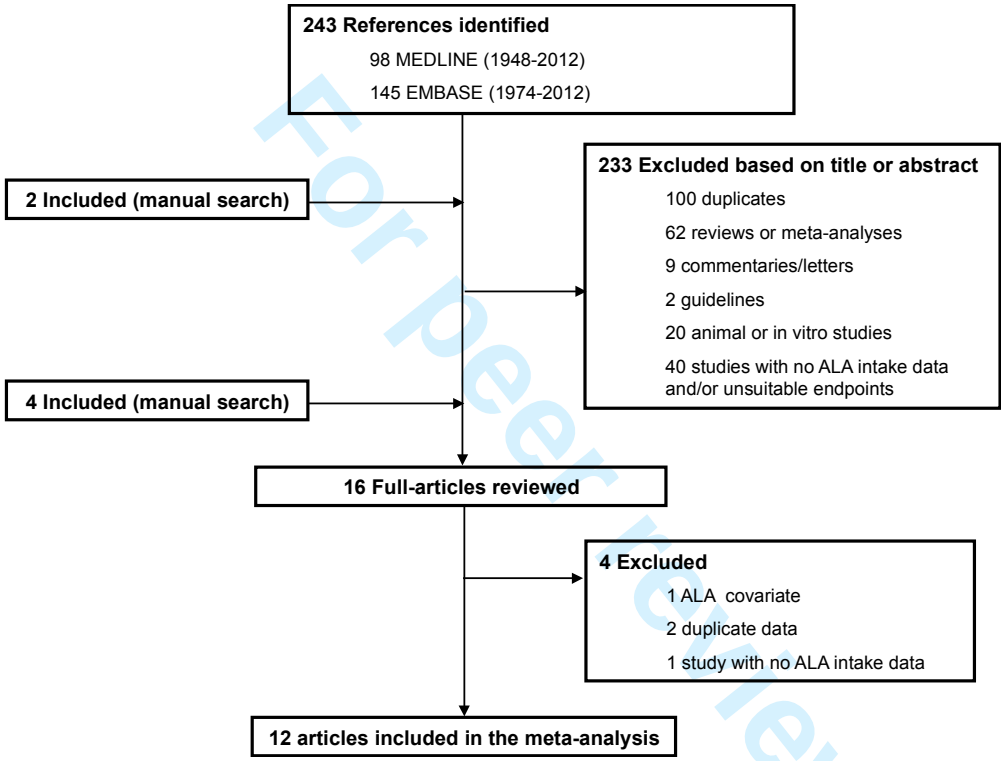

Figure 1 - Flow of the literature.

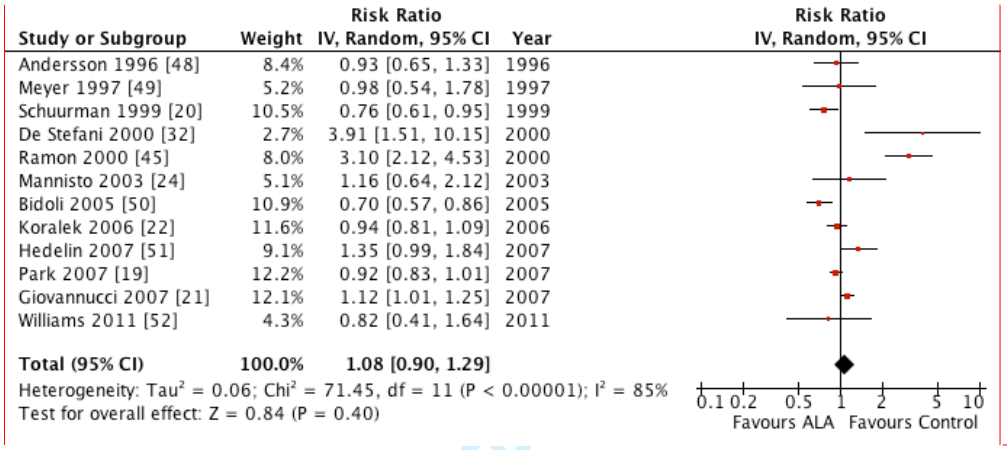

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

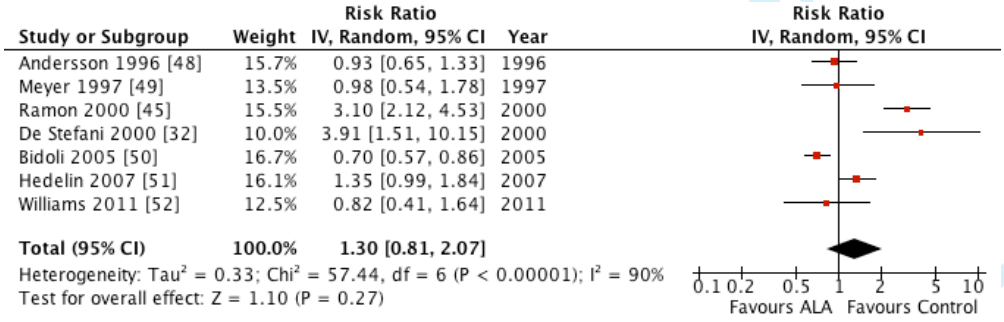

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a

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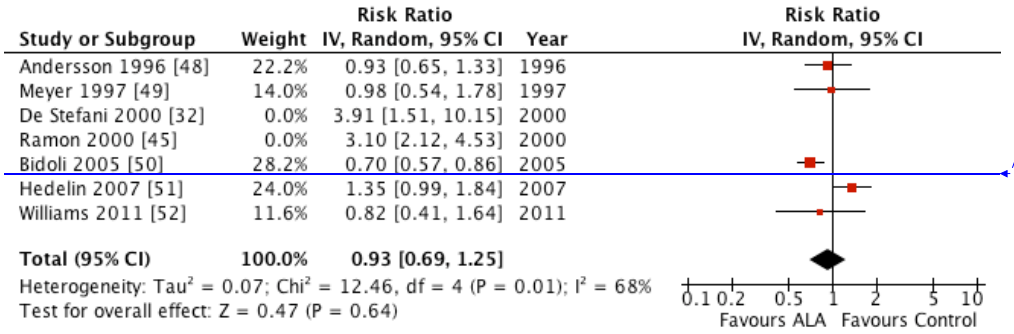

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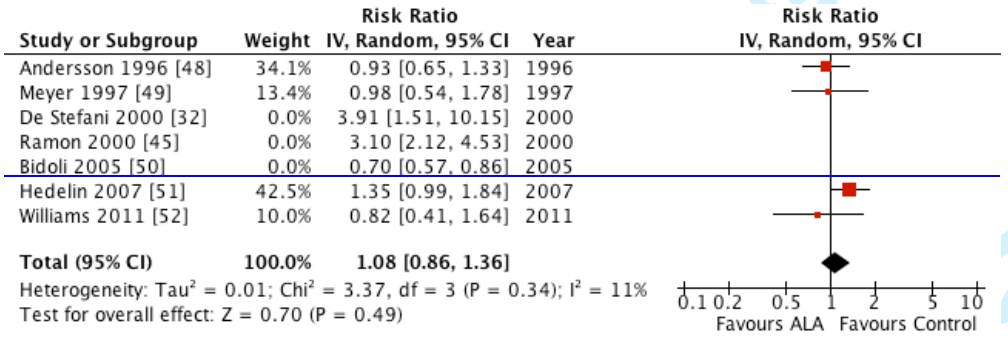
~~**Figure 4 –** Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by De Stefani et al.[32] and Ramon et al.[45] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity[55].~~



~~**Figure 5 –** Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies after the removal of the studies by De Stefani et al.[32], Ramon et al.[45], and Bidoli et al.[50] and following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects~~

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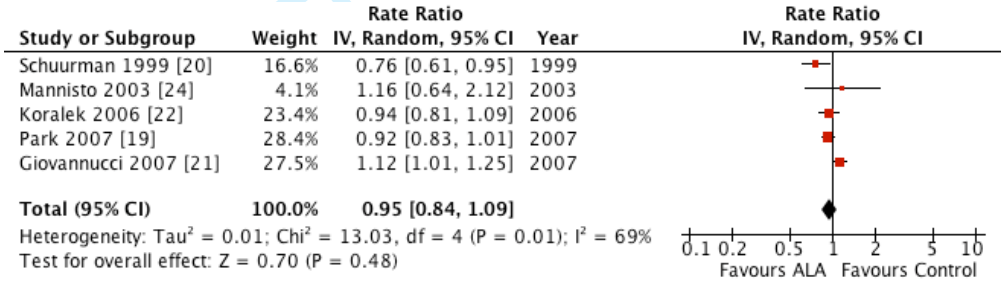

Figure 46 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

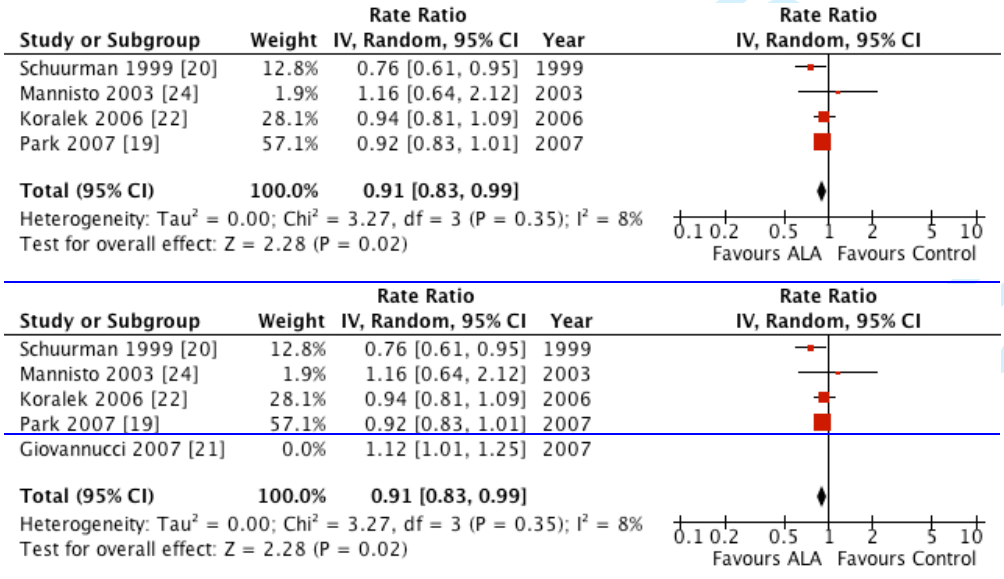

Figure 57 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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Table 1 - Characteristics of studies included in the meta-analysis of alpha-linolenic acid intake and prostate cancer

Study	Country of Origin	Study Design	Sample size	Age (years)	Incident Cases	Follow-up (years)	Biomarker level (unit)	Relative Risk or Odds Ratio	95% CI
Andersson et al. 1996 [39]	Sweden	Case-control	626 cases/536 controls	<80	-	-	0.917 - 1.362	0.93	0.65-1.32
Meyer et al. 1997 [36]	Canada	Case-control	216 cases/583 controls	≥65	-	-	-	0.98	0.54-1.78
Schuurman et al. 1999 [12]*	Netherlands	Nested case-cohort	58,279 (1535 subcohort)	55-69	642	6.3	0.7 - 2.1	0.78	0.56-1.04
De Stefani et al. 2000 [38]	Uruguay	Case-control	217 cases/431 controls	40-89	-	-	≤0.8 - ≥1.5	3.91	1.60-10.1
Ramon et al. 2000 [40]	Spain	Case-control	217 cases/434 controls	<65-80	-	-	0.72 - 2.1	3.1	2.2-4.7
Mannisto et al. 2003 [22]*	Finland	Nested case-control	198 cases/198 controls	50-69	346	6-8	1.0 - 2.8	1.18	0.64-2.19
Bidoli et al. 2005 [41]	Italy	Case-control	1294 cases/1451 controls	48-74	-	-	mean 1.6	0.7	0.6-0.8
Kesplak et al. 2006 [20]*	United States	Prospective cohort	29,892	55-74	1698	6.1	1.08 - 1.75	0.94	0.81-1.09
Hedelin et al. 2007 [42]	Sweden	Case-control	1499 cases/1130 controls	mean 67.5	-	-	0.08 - 0.60	1.38	0.99-1.94
Giovannucci et al. 2007 [16]*	United States	Prospective cohort	47,780	40-75	3644	16	<0.78 - ≥1.52	1.12	1.01-1.25
Park et al. 2007 [17]*	United States	Prospective cohort	82,465	≥45	4404	8	1.1 - 2.14†	0.88	0.84-1.02
Williams et al. 2011 [43]	United States	Case-control	79 cases/187 controls	≥18	-	-	≤1.5 - 4.15††	0.58	0.41-1.85

* Prospective studies.
† Based on a 2000 kcal diet.

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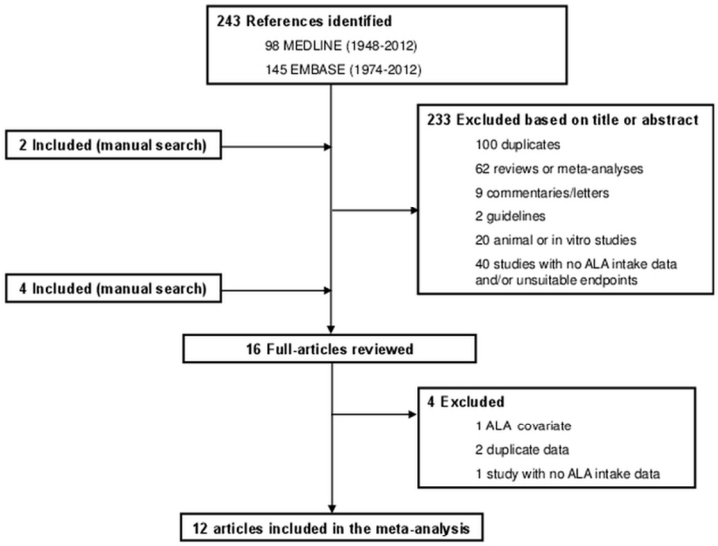

Figure 1 - Flow of the literature.

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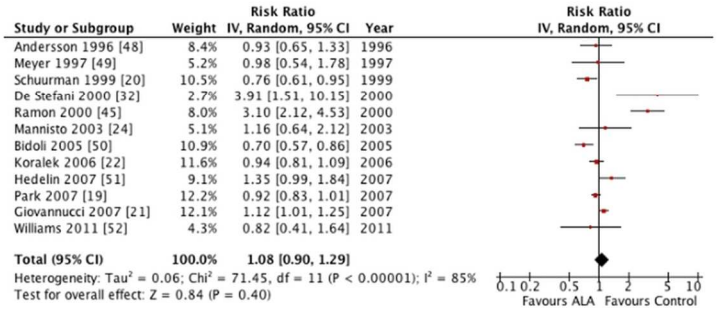

Figure 2 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control, nested case-control, nested case-cohort, and cohort studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of P<0.10 and quantified by I^2, where $I^2 \geq 50$ % is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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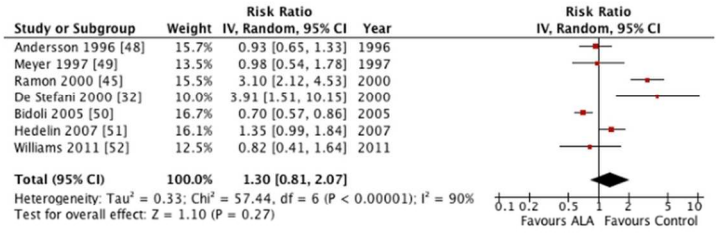

Figure 3 – Pooled effect of dietary ALA intake on prostate cancer risk in case-control studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50\%$ is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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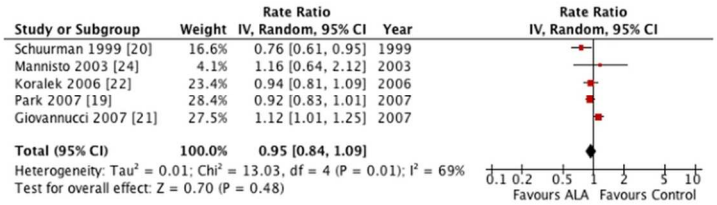

Figure 4 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50\%$ is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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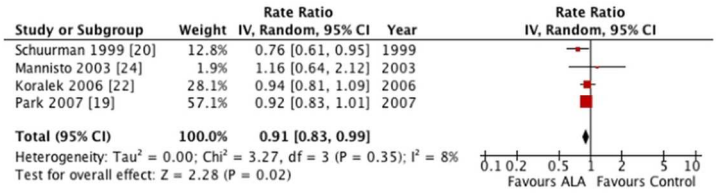

Figure 5 – Pooled effect of dietary ALA intake on prostate cancer risk in prospective studies after the systematic removal of the study by Giovannucci et al. [21] following a sensitivity analysis. Relative Risk (RR) with 95% CI, study weights, and pooled effect estimates were generated using the general inverse variance method with random effects models (RevMan 5.1, Cochrane Library software, Oxford, UK). Inter-study heterogeneity was tested by Cochrane's Q (Chi^2) at a significance level of $P<0.10$ and quantified by I^2, where $I^2 \geq 50\,\%$ is considered to be evidence of substantial heterogeneity and ≥75%, considerable heterogeneity [55].

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